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TITLE OF THE INVENTION (500 characters max)

FLJ10607 AS MODIFIER OF THE AXIN PATHWAY AND METHODS OF USE

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ Application Data Sheet. See 37 CFR 1.76

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☐ Applicant claims small entity status. See 37 CFR 1.27.

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☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____.

Respectfully submitted,

SIGNATURE _____ Date 12/30/02

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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PROVISIONAL APPLICATION COVER SHEET
Additional Page

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Docket Number	EX02-147P

INVENTOR(S)/APPLICANT(S)

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Number 1 of 1

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EX02-147P

FLJ10607 AS MODIFIER OF THE AXIN PATHWAY AND METHODS OF USE

BACKGROUND OF THE INVENTION

Deregulation of beta-catenin signaling is a frequent and early event in the development of a variety of human tumors, including colon cancer, melanoma, ovarian cancer, and prostate cancer. Activation of beta-catenin signaling can occur in tumor cells by loss-of-function mutations in the tumor suppressor genes Axin or APC, as well as by gain-of-function mutations in the oncogene beta-catenin itself. Axin normally functions as a scaffolding protein that binds beta-catenin, APC, and the serine/threonine kinase GSK3-beta. Assembly of this degradation complex allows GSK3-beta to phosphorylate beta-catenin, which leads to beta-catenin ubiquitination and degradation by the proteasome. In the absence of Axin activity, beta-catenin protein becomes stabilized and accumulates in the nucleus where it acts as a transcriptional co-activator with TCF for the induction of target genes, including the cell cycle regulators cyclin D1 and c-Myc.

The *C. elegans* gene *pry-1* is the structural and functional ortholog of vertebrate Axin (Korswagen HC et al. (2002) Genes Dev. 16:1291-302). PRY-1 is predicted to contain conserved RGS and DIX domains that, in Axin, bind APC and Dishevelled, respectively. Overexpression of the *C. elegans pry-1* gene in zebrafish can fully rescue the mutant phenotype of *masterblind*, the zebrafish Axin1 mutation. *pry-1* loss-of-function mutations produce several phenotypes that appear to result from increased beta-catenin signaling (Gleason JE et al. (2002) Genes Dev. 16:1281-90; Korswagen et al., *supra*).

The ability to manipulate the genomes of model organisms such as *C. elegans* provides a powerful means to analyze biochemical processes that, due to significant evolutionary conservation, have direct relevance to more complex vertebrate organisms. Due to a high level of gene and pathway conservation, the strong similarity of cellular processes, and the functional conservation of genes between these model organisms and mammals, identification of the involvement of novel genes in particular pathways and their functions in such model organisms can directly contribute to the understanding of the correlative pathways and methods of modulating them in mammals (see, for example, Dulubova I, et al, J Neurochem 2001

Apr;77(1):229-38; Cai T, et al., Diabetologia 2001 Jan;44(1):81-8; Pasquinelli AE, et al., Nature. 2000 Nov 2;408(6808):37-8; Ivanov IP, et al., EMBO J 2000 Apr 17;19(8):1907-17; Vajo Z et al., Mamm Genome 1999 Oct;10(10):1000-4). For example, a genetic screen can be carried out in an invertebrate model organism having underexpression (e.g. knockout) or overexpression of a gene (referred to as a "genetic entry point") that yields a visible phenotype. Additional genes are mutated in a random or targeted manner. When a gene mutation changes the original phenotype caused by the mutation in the genetic entry point, the gene is identified as a "modifier" involved in the same or overlapping pathway as the genetic entry point. When the genetic entry point is an ortholog of a human gene implicated in a disease pathway, such as axin, modifier genes can be identified that may be attractive candidate targets for novel therapeutics.

All references cited herein, including patents, patent applications, publications, and sequence information in referenced Genbank identifier numbers, are incorporated herein in their entireties.

SUMMARY OF THE INVENTION

We have discovered genes that modify the axin pathway in *C. elegans*, and identified their human orthologs, hereinafter referred to as FLJ10607. The invention provides methods for utilizing these axin modifier genes and polypeptides to identify FLJ10607-modulating agents that are candidate therapeutic agents that can be used in the treatment of disorders associated with defective or impaired axin function and/or FLJ10607 function. Preferred FLJ10607-modulating agents specifically bind to FLJ10607 polypeptides and restore axin function. Other preferred FLJ10607-modulating agents are nucleic acid modulators such as antisense oligomers and RNAi that repress FLJ10607 gene expression or product activity by, for example, binding to and inhibiting the respective nucleic acid (i.e. DNA or mRNA).

FLJ10607 modulating agents may be evaluated by any convenient *in vitro* or *in vivo* assay for molecular interaction with an FLJ10607 polypeptide or nucleic acid. In one embodiment, candidate FLJ10607 modulating agents are tested with an assay system comprising a FLJ10607 polypeptide or nucleic acid. Agents that produce a

change in the activity of the assay system relative to controls are identified as candidate axin modulating agents. The assay system may be cell-based or cell-free. FLJ10607-modulating agents include FLJ10607 related proteins (e.g. dominant negative mutants, and biotherapeutics); FLJ10607 -specific antibodies; FLJ10607 -specific antisense oligomers and other nucleic acid modulators; and chemical agents that specifically bind to or interact with FLJ10607 or compete with FLJ10607 binding partner (e.g. by binding to an FLJ10607 binding partner). In one specific embodiment, a small molecule modulator is identified using an enzymatic assay. In specific embodiments, the screening assay system is selected from a binding assay, an apoptosis assay, a cell proliferation assay, an angiogenesis assay, and a hypoxic induction assay.

In another embodiment, candidate axin pathway modulating agents are further tested using a second assay system that detects changes in the axin pathway, such as angiogenic, apoptotic, or cell proliferation changes produced by the originally identified candidate agent or an agent derived from the original agent. The second assay system may use cultured cells or non-human animals. In specific embodiments, the secondary assay system uses non-human animals, including animals predetermined to have a disease or disorder implicating the axin pathway, such as an angiogenic, apoptotic, or cell proliferation disorder (e.g. cancer).

The invention further provides methods for modulating the FLJ10607 function and/or the axin pathway in a mammalian cell by contacting the mammalian cell with an agent that specifically binds a FLJ10607 polypeptide or nucleic acid. The agent may be a small molecule modulator, a nucleic acid modulator, or an antibody and may be administered to a mammalian animal predetermined to have a pathology associated the axin pathway.

DETAILED DESCRIPTION OF THE INVENTION

Genetic screens were designed to identify modifiers of the axin pathway in *C. elegans,* where a reduction of function pry-1 (axin) mutant was used. Various specific genes were silenced by RNA inhibition (RNAi). Methods for using RNAi to silence genes in *C. elegans* are known in the art (Fire A, et al., 1998 Nature

391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); WO9932619). Genes causing altered phenotypes in the worms were identified as modifiers of the axin pathway. Accordingly, vertebrate orthologs of these modifiers, and preferably the human orthologs, FLJ10607 genes (i.e., nucleic acids and polypeptides) are attractive drug targets for the treatment of pathologies associated with a defective axin signaling pathway, such as cancer.

In vitro and in vivo methods of assessing FLJ10607 function are provided herein. Modulation of the FLJ10607 or their respective binding partners is useful for understanding the association of the axin pathway and its members in normal and disease conditions and for developing diagnostics and therapeutic modalities for axin related pathologies. FLJ10607-modulating agents that act by inhibiting or enhancing FLJ10607 expression, directly or indirectly, for example, by affecting an FLJ10607 function such as enzymatic (e.g., catalytic) or binding activity, can be identified using methods provided herein. FLJ10607 modulating agents are useful in diagnosis, therapy and pharmaceutical development.

Nucleic acids and polypeptides of the invention

Sequences related to FLJ10607 nucleic acids and polypeptides that can be used in the invention are disclosed in Genbank (referenced by Genbank identifier (GI) number) as GI# 18597005 (SEQ ID NO:1) for nucleic acid, and GI# 18597006 (SEQ ID NO:2) for polypeptide.

The term "FLJ10607 polypeptide" refers to a full-length FLJ10607 protein or a functionally active fragment or derivative thereof. A "functionally active" FLJ10607 fragment or derivative exhibits one or more functional activities associated with a full-length, wild-type FLJ10607 protein, such as antigenic or immunogenic activity, enzymatic activity, ability to bind natural cellular substrates, etc. The functional activity of FLJ10607 proteins, derivatives and fragments can be assayed by various methods known to one skilled in the art (Current Protocols in Protein Science (1998) Coligan *et al.*, eds., John Wiley & Sons, Inc., Somerset, New Jersey) and as further discussed below. In one embodiment, a functionally active FLJ10607 polypeptide is a FLJ10607 derivative capable of rescuing defective endogenous FLJ10607 activity,

such as in cell based or animal assays; the rescuing derivative may be from the same or a different species. For purposes herein, functionally active fragments also include those fragments that comprise one or more structural domains of an FLJ10607, such as a a binding domain. Protein domains can be identified using the PFAM program (Bateman A., et al., Nucleic Acids Res, 1999, 27:260-2). Methods for obtaining FLJ10607 polypeptides are also further described below. In some embodiments, preferred fragments are functionally active, domain-containing fragments comprising at least 25 contiguous amino acids, preferably at least 50, more preferably 75, and most preferably at least 100 contiguous amino acids of FLJ10607. In further preferred embodiments, the fragment comprises the entire functionally active domain.

The term "FLJ10607 nucleic acid" refers to a DNA or RNA molecule that encodes a FLJ10607 polypeptide. Preferably, the FLJ10607 polypeptide or nucleic acid or fragment thereof is from a human, but can also be an ortholog, or derivative thereof with at least 70% sequence identity, preferably at least 80%, more preferably 85%, still more preferably 90%, and most preferably at least 95% sequence identity with human FLJ10607. Methods of identifying orthlogs are known in the art. Normally, orthologs in different species retain the same function, due to presence of one or more protein motifs and/or 3-dimensional structures. Orthologs are generally identified by sequence homology analysis, such as BLAST analysis, usually using protein bait sequences. Sequences are assigned as a potential ortholog if the best hit sequence from the forward BLAST result retrieves the original query sequence in the reverse BLAST (Huynen MA and Bork P, Proc Natl Acad Sci (1998) 95:5849-5856; Huynen MA *et al.*, Genome Research (2000) 10:1204-1210). Programs for multiple sequence alignment, such as CLUSTAL (Thompson JD et al, 1994, Nucleic Acids Res 22:4673-4680) may be used to highlight conserved regions and/or residues of orthologous proteins and to generate phylogenetic trees. In a phylogenetic tree representing multiple homologous sequences from diverse species (e.g., retrieved through BLAST analysis), orthologous sequences from two species generally appear closest on the tree with respect to all other sequences from these two species. Structural threading or other analysis of protein folding (e.g., using software by ProCeryon, Biosciences, Salzburg, Austria) may also identify potential orthologs. In

evolution, when a gene duplication event follows speciation, a single gene in one species, such as *C. elegans*, may correspond to multiple genes (paralogs) in another, such as human. As used herein, the term "orthologs" encompasses paralogs. As used herein, "percent (%) sequence identity" with respect to a subject sequence, or a specified portion of a subject sequence, is defined as the percentage of nucleotides or amino acids in the candidate derivative sequence identical with the nucleotides or amino acids in the subject sequence (or specified portion thereof), after aligning the sequences and introducing gaps, if necessary to achieve the maximum percent sequence identity, as generated by the program WU-BLAST-2.0a19 (Altschul *et al.*, J. Mol. Biol. (1997) 215:403-410) with all the search parameters set to default values. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched. A % identity value is determined by the number of matching identical nucleotides or amino acids divided by the sequence length for which the percent identity is being reported. "Percent (%) amino acid sequence similarity" is determined by doing the same calculation as for determining % amino acid sequence identity, but including conservative amino acid substitutions in addition to identical amino acids in the computation.

A conservative amino acid substitution is one in which an amino acid is substituted for another amino acid having similar properties such that the folding or activity of the protein is not significantly affected. Aromatic amino acids that can be substituted for each other are phenylalanine, tryptophan, and tyrosine; interchangeable hydrophobic amino acids are leucine, isoleucine, methionine, and valine; interchangeable polar amino acids are glutamine and asparagine; interchangeable basic amino acids are arginine, lysine and histidine; interchangeable acidic amino acids are aspartic acid and glutamic acid; and interchangeable small amino acids are alanine, serine, threonine, cysteine and glycine.

Alternatively, an alignment for nucleic acid sequences is provided by the local homology algorithm of Smith and Waterman (Smith and Waterman, 1981, Advances in Applied Mathematics 2:482-489; database: European Bioinformatics Institute;

Smith and Waterman, 1981, J. of Molec.Biol., 147:195-197; Nicholas et al., 1998, "A Tutorial on Searching Sequence Databases and Sequence Scoring Methods" (www.psc.edu) and references cited therein.; W.R. Pearson, 1991, Genomics 11:635-650). This algorithm can be applied to amino acid sequences by using the scoring matrix developed by Dayhoff (Dayhoff: Atlas of Protein Sequences and Structure, M. O. Dayhoff ed., 5 suppl. 3:353-358, National Biomedical Research Foundation, Washington, D.C., USA), and normalized by Gribskov (Gribskov 1986 Nucl. Acids Res. 14(6):6745-6763). The Smith-Waterman algorithm may be employed where default parameters are used for scoring (for example, gap open penalty of 12, gap extension penalty of two). From the data generated, the "Match" value reflects "sequence identity."

Derivative nucleic acid molecules of the subject nucleic acid molecules include sequences that hybridize to the nucleic acid sequence of SEQ ID NO:1. The stringency of hybridization can be controlled by temperature, ionic strength, pH, and the presence of denaturing agents such as formamide during hybridization and washing. Conditions routinely used are set out in readily available procedure texts (*e.g.*, Current Protocol in Molecular Biology, Vol. 1, Chap. 2.10, John Wiley & Sons, Publishers (1994); Sambrook *et al.*, Molecular Cloning, Cold Spring Harbor (1989)). In some embodiments, a nucleic acid molecule of the invention is capable of hybridizing to a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO:1 under high stringency hybridization conditions that are: prehybridization of filters containing nucleic acid for 8 hours to overnight at 65° C in a solution comprising 6X single strength citrate (SSC) (1X SSC is 0.15 M NaCl, 0.015 M Na citrate; pH 7.0), 5X Denhardt's solution, 0.05% sodium pyrophosphate and 100 μg/ml herring sperm DNA; hybridization for 18-20 hours at 65° C in a solution containing 6X SSC, 1X Denhardt's solution, 100 μg/ml yeast tRNA and 0.05% sodium pyrophosphate; and washing of filters at 65° C for 1h in a solution containing 0.1X SSC and 0.1% SDS (sodium dodecyl sulfate).

In other embodiments, moderately stringent hybridization conditions are used that are: pretreatment of filters containing nucleic acid for 6 h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.1%

PVP, 0.1% Ficoll, 1% BSA, and 500 μg/ml denatured salmon sperm DNA; hybridization for 18-20h at 40° C in a solution containing 35% formamide, 5X SSC, 50 mM Tris-HCl (pH7.5), 5mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.2% BSA, 100 μg/ml salmon sperm DNA, and 10% (wt/vol) dextran sulfate; followed by washing twice for 1 hour at 55° C in a solution containing 2X SSC and 0.1% SDS.

Alternatively, low stringency conditions can be used that are: incubation for 8 hours to overnight at 37° C in a solution comprising 20% formamide, 5 x SSC, 50 mM sodium phosphate (pH 7.6), 5X Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured sheared salmon sperm DNA; hybridization in the same buffer for 18 to 20 hours; and washing of filters in 1 x SSC at about 37° C for 1 hour.

Isolation, Production, Expression, and Mis-expression of FLJ10607 Nucleic Acids and Polypeptides

FLJ10607 nucleic acids and polypeptides, useful for identifying and testing agents that modulate FLJ10607 function and for other applications related to the involvement of FLJ10607 in the axin pathway. FLJ10607 nucleic acids and derivatives and orthologs thereof may be obtained using any available method. For instance, techniques for isolating cDNA or genomic DNA sequences of interest by screening DNA libraries or by using polymerase chain reaction (PCR) are well known in the art. In general, the particular use for the protein will dictate the particulars of expression, production, and purification methods. For instance, production of proteins for use in screening for modulating agents may require methods that preserve specific biological activities of these proteins, whereas production of proteins for antibody generation may require structural integrity of particular epitopes. Expression of proteins to be purified for screening or antibody production may require the addition of specific tags (*e.g.*, generation of fusion proteins). Overexpression of an FLJ10607 protein for assays used to assess FLJ10607 function, such as involvement in cell cycle regulation or hypoxic response, may require expression in eukaryotic cell lines capable of these cellular activities. Techniques for the expression, production, and purification of proteins are well known in the art; any suitable means therefore may be used (e.g., Higgins SJ and Hames BD (eds.) Protein

Expression: A Practical Approach, Oxford University Press Inc., New York 1999; Stanbury PF et al., Principles of Fermentation Technology, 2nd edition, Elsevier Science, New York, 1995; Doonan S (ed.) Protein Purification Protocols, Humana Press, New Jersey, 1996; Coligan JE et al, Current Protocols in Protein Science (eds.), 1999, John Wiley & Sons, New York). In particular embodiments, recombinant FLJ10607 is expressed in a cell line known to have defective axin function. The recombinant cells are used in cell-based screening assay systems of the invention, as described further below.

The nucleotide sequence encoding an FLJ10607 polypeptide can be inserted into any appropriate expression vector. The necessary transcriptional and translational signals, including promoter/enhancer element, can derive from the native FLJ10607 gene and/or its flanking regions or can be heterologous. A variety of host-vector expression systems may be utilized, such as mammalian cell systems infected with virus (*e.g.* vaccinia virus, adenovirus, *etc.*); insect cell systems infected with virus (*e.g.* baculovirus); microorganisms such as yeast containing yeast vectors, or bacteria transformed with bacteriophage, plasmid, or cosmid DNA. An isolated host cell strain that modulates the expression of, modifies, and/or specifically processes the gene product may be used.

To detect expression of the FLJ10607 gene product, the expression vector can comprise a promoter operably linked to an FLJ10607 gene nucleic acid, one or more origins of replication, and, one or more selectable markers (*e.g.* thymidine kinase activity, resistance to antibiotics, *etc.*). Alternatively, recombinant expression vectors can be identified by assaying for the expression of the FLJ10607 gene product based on the physical or functional properties of the FLJ10607 protein in *in vitro* assay systems (*e.g.* immunoassays).

The FLJ10607 protein, fragment, or derivative may be optionally expressed as a fusion, or chimeric protein product (i.e. it is joined via a peptide bond to a heterologous protein sequence of a different protein), for example to facilitate purification or detection. A chimeric product can be made by ligating the appropriate nucleic acid sequences encoding the desired amino acid sequences to each other using standard methods and expressing the chimeric product. A chimeric product may also

be made by protein synthetic techniques, *e.g.* by use of a peptide synthesizer (Hunkapiller *et al.*, Nature (1984) 310:105-111).

Once a recombinant cell that expresses the FLJ10607 gene sequence is identified, the gene product can be isolated and purified using standard methods (*e.g.* ion exchange, affinity, and gel exclusion chromatography; centrifugation; differential solubility; electrophoresis). Alternatively, native FLJ10607 proteins can be purified from natural sources, by standard methods (*e.g.* immunoaffinity purification). Once a protein is obtained, it may be quantified and its activity measured by appropriate methods, such as immunoassay, bioassay, or other measurements of physical properties, such as crystallography.

The methods of this invention may also use cells that have been engineered for altered expression (mis-expression) of FLJ10607 or other genes associated with the axin pathway. As used herein, mis-expression encompasses ectopic expression, over-expression, under-expression, and non-expression (*e.g.* by gene knock-out or blocking expression that would otherwise normally occur).

Genetically modified animals

Animal models that have been genetically modified to alter FLJ10607 expression may be used in *in vivo* assays to test for activity of a candidate axin modulating agent, or to further assess the role of FLJ10607 in a axin pathway process such as apoptosis or cell proliferation. Preferably, the altered FLJ10607 expression results in a detectable phenotype, such as decreased or increased levels of cell proliferation, angiogenesis, or apoptosis compared to control animals having normal FLJ10607 expression. The genetically modified animal may additionally have altered axin expression (e.g. axin knockout). Preferred genetically modified animals are mammals such as primates, rodents (preferably mice or rats), among others. Preferred non-mammalian species include zebrafish, *C. elegans*, and *Drosophila*. Preferred genetically modified animals are transgenic animals having a heterologous nucleic acid sequence present as an extrachromosomal element in a portion of its cells, i.e. mosaic animals (see, for example, techniques described by Jakobovits, 1994, Curr. Biol. 4:761-763.) or stably integrated into its germ line DNA (i.e., in the

genomic sequence of most or all of its cells). Heterologous nucleic acid is introduced into the germ line of such transgenic animals by genetic manipulation of, for example, embryos or embryonic stem cells of the host animal.

Methods of making transgenic animals are well-known in the art (for transgenic mice see Brinster et al., Proc. Nat. Acad. Sci. USA 82: 4438-4442 (1985), U.S. Pat. Nos. 4,736,866 and 4,870,009, both by Leder et al., U.S. Pat. No. 4,873,191 by Wagner et al., and Hogan, B., Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); for particle bombardment see U.S. Pat. No., 4,945,050, by Sandford *et al.*; for transgenic *Drosophila* see Rubin and Spradling, Science (1982) 218:348-53 and U.S. Pat. No. 4,670,388; for transgenic insects see Berghammer A.J. *et al.*, A Universal Marker for Transgenic Insects (1999) Nature 402:370-371; for transgenic Zebrafish see Lin S., Transgenic Zebrafish, Methods Mol Biol. (2000);136:375-3830); for microinjection procedures for fish, amphibian eggs and birds see Houdebine and Chourrout, Experientia (1991) 47:897-905; for transgenic rats see Hammer *et al.*, Cell (1990) 63:1099-1112; and for culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection see, e.g., Teratocarcinomas and Embryonic Stem Cells, A Practical Approach, E. J. Robertson, ed., IRL Press (1987)). Clones of the nonhuman transgenic animals can be produced according to available methods (see Wilmut, I. *et al.* (1997) Nature 385:810-813; and PCT International Publication Nos. WO 97/07668 and WO 97/07669).

In one embodiment, the transgenic animal is a "knock-out" animal having a heterozygous or homozygous alteration in the sequence of an endogenous FLJ10607 gene that results in a decrease of FLJ10607 function, preferably such that FLJ10607 expression is undetectable or insignificant. Knock-out animals are typically generated by homologous recombination with a vector comprising a transgene having at least a portion of the gene to be knocked out. Typically a deletion, addition or substitution has been introduced into the transgene to functionally disrupt it. The transgene can be a human gene (e.g., from a human genomic clone) but more preferably is an ortholog of the human gene derived from the transgenic host species.

For example, a mouse FLJ10607 gene is used to construct a homologous recombination vector suitable for altering an endogenous FLJ10607 gene in the mouse genome. Detailed methodologies for homologous recombination in mice are available (see Capecchi, Science (1989) 244:1288-1292; Joyner *et al.*, Nature (1989) 338:153-156). Procedures for the production of non-rodent transgenic mammals and other animals are also available (Houdebine and Chourrout, *supra*; Pursel *et al.*, Science (1989) 244:1281-1288; Simms *et al.*, Bio/Technology (1988) 6:179-183). In a preferred embodiment, knock-out animals, such as mice harboring a knockout of a specific gene, may be used to produce antibodies against the human counterpart of the gene that has been knocked out (Claesson MH et al., (1994) Scan J Immunol 40:257-264; Declerck PJ et al., (1995) J Biol Chem. 270:8397-400).

In another embodiment, the transgenic animal is a "knock-in" animal having an alteration in its genome that results in altered expression (e.g., increased (including ectopic) or decreased expression) of the FLJ10607 gene, e.g., by introduction of additional copies of FLJ10607, or by operatively inserting a regulatory sequence that provides for altered expression of an endogenous copy of the FLJ10607 gene. Such regulatory sequences include inducible, tissue-specific, and constitutive promoters and enhancer elements. The knock-in can be homozygous or heterozygous.

Transgenic nonhuman animals can also be produced that contain selected systems allowing for regulated expression of the transgene. One example of such a system that may be produced is the cre/loxP recombinase system of bacteriophage P1 (Lakso *et al.*, PNAS (1992) 89:6232-6236; U.S. Pat. No. 4,959,317). If a cre/loxP recombinase system is used to regulate expression of the transgene, animals containing transgenes encoding both the Cre recombinase and a selected protein are required. Such animals can be provided through the construction of "double" transgenic animals, e.g., by mating two transgenic animals, one containing a transgene encoding a selected protein and the other containing a transgene encoding a recombinase. Another example of a recombinase system is the FLP recombinase system of Saccharomyces cerevisiae (O'Gorman et al. (1991) Science 251:1351-1355; U.S. Pat. No. 5,654,182). In a preferred embodiment, both Cre-LoxP and Flp-Frt are used in the same system to regulate expression of the

transgene, and for sequential deletion of vector sequences in the same cell (Sun X et al (2000) Nat Genet 25:83-6).

The genetically modified animals can be used in genetic studies to further elucidate the axin pathway, as animal models of disease and disorders implicating defective axin function, and for *in vivo* testing of candidate therapeutic agents, such as those identified in screens described below. The candidate therapeutic agents are administered to a genetically modified animal having altered FLJ10607 function and phenotypic changes are compared with appropriate control animals such as genetically modified animals that receive placebo treatment, and/or animals with unaltered FLJ10607 expression that receive candidate therapeutic agent.

In addition to the above-described genetically modified animals having altered FLJ10607 function, animal models having defective axin function (and otherwise normal FLJ10607 function), can be used in the methods of the present invention. For example, a axin knockout mouse can be used to assess, *in vivo*, the activity of a candidate axin modulating agent identified in one of the *in vitro* assays described below. Preferably, the candidate axin modulating agent when administered to a model system with cells defective in axin function, produces a detectable phenotypic change in the model system indicating that the axin function is restored, for example, the cells exhibit normal cell cycle progression.

Modulating Agents

The invention provides methods to identify agents that interact with and/or modulate the function of FLJ10607 and/or the axin pathway. Modulating agents identified by the methods are also part of the invention. Such agents are useful in a variety of diagnostic and therapeutic applications associated with the axin pathway, as well as in further analysis of the FLJ10607 protein and its contribution to the axin pathway. Accordingly, the invention also provides methods for modulating the axin pathway comprising the step of specifically modulating FLJ10607 activity by administering a FLJ10607-interacting or -modulating agent.

As used herein, an "FLJ10607-modulating agent" is any agent that modulates FLJ10607 function, for example, an agent that interacts with FLJ10607 to inhibit or

enhance FLJ10607 activity or otherwise affect normal FLJ10607 function. FLJ10607 function can be affected at any level, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In a preferred embodiment, the FLJ10607 - modulating agent specifically modulates the function of the FLJ10607. The phrases "specific modulating agent", "specifically modulates", etc., are used herein to refer to modulating agents that directly bind to the FLJ10607 polypeptide or nucleic acid, and preferably inhibit, enhance, or otherwise alter, the function of the FLJ10607. These phrases also encompasses modulating agents that alter the interaction of the FLJ10607 with a binding partner, substrate, or cofactor (e.g. by binding to a binding partner of an FLJ10607, or to a protein/binding partner complex, and altering FLJ10607 function). In a further preferred embodiment, the FLJ10607- modulating agent is a modulator of the axin pathway (e.g. it restores and/or upregulates axin function) and thus is also a axin-modulating agent.

Preferred FLJ10607-modulating agents include small molecule compounds; FLJ10607-interacting proteins, including antibodies and other biotherapeutics; and nucleic acid modulators such as antisense and RNA inhibitors. The modulating agents may be formulated in pharmaceutical compositions, for example, as compositions that may comprise other active ingredients, as in combination therapy, and/or suitable carriers or excipients. Techniques for formulation and administration of the compounds may be found in "Remington's Pharmaceutical Sciences" Mack Publishing Co., Easton, PA, 19th edition.

Small molecule modulators

Small molecules are often preferred to modulate function of proteins with enzymatic function, and/or containing protein interaction domains. Chemical agents, referred to in the art as "small molecule" compounds are typically organic, non-peptide molecules, having a molecular weight less than 10,000, preferably less than 5,000, more preferably less than 1,000, and most preferably less than 500. This class of modulators includes chemically synthesized molecules, for instance, compounds from combinatorial chemical libraries. Synthetic compounds may be rationally designed or identified based on known or inferred properties of the FLJ10607 protein or may be identified by screening

compound libraries. Alternative appropriate modulators of this class are natural products, particularly secondary metabolites from organisms such as plants or fungi, which can also be identified by screening compound libraries for FLJ10607–modulating activity. Methods for generating and obtaining compounds are well known in the art (Schreiber SL, Science (2000) 151: 1964-1969; Radmann J and Gunther J, Science (2000) 151:1947-1948).

Small molecule modulators identified from screening assays, as described below, can be used as lead compounds from which candidate clinical compounds may be designed, optimized, and synthesized. Such clinical compounds may have utility in treating pathologies associated with the axin pathway. The activity of candidate small molecule modulating agents may be improved several-fold through iterative secondary functional validation, as further described below, structure determination, and candidate modulator modification and testing. Additionally, candidate clinical compounds are generated with specific regard to clinical and pharmacological properties. For example, the reagents may be derivatized and re-screened using *in vitro* and *in vivo* assays to optimize activity and minimize toxicity for pharmaceutical development.

Protein Modulators

Specific FLJ10607-interacting proteins are useful in a variety of diagnostic and therapeutic applications related to the axin pathway and related disorders, as well as in validation assays for other FLJ10607-modulating agents. In a preferred embodiment, FLJ10607-interacting proteins affect normal FLJ10607 function, including transcription, protein expression, protein localization, and cellular or extra-cellular activity. In another embodiment, FLJ10607-interacting proteins are useful in detecting and providing information about the function of FLJ10607 proteins, as is relevant to axin related disorders, such as cancer (e.g., for diagnostic means).

An FLJ10607-interacting protein may be endogenous, i.e. one that naturally interacts genetically or biochemically with an FLJ10607, such as a member of the FLJ10607 pathway that modulates FLJ10607 expression, localization, and/or activity. FLJ10607-modulators include dominant negative forms of FLJ10607-interacting proteins and of FLJ10607 proteins themselves. Yeast two-hybrid and variant screens offer preferred

methods for identifying endogenous FLJ10607-interacting proteins (Finley, R. L. et al. (1996) in DNA Cloning-Expression Systems: A Practical Approach, eds. Glover D. & Hames B. D (Oxford University Press, Oxford, England), pp. 169-203; Fashema SF et al., Gene (2000) 250:1-14; Drees BL Curr Opin Chem Biol (1999) 3:64-70; Vidal M and Legrain P Nucleic Acids Res (1999) 27:919-29; and U.S. Pat. No. 5,928,868). Mass spectrometry is an alternative preferred method for the elucidation of protein complexes (reviewed in, e.g., Pandley A and Mann M, Nature (2000) 405:837-846; Yates JR 3rd, Trends Genet (2000) 16:5-8).

An FLJ10607-interacting protein may be an exogenous protein, such as an FLJ10607-specific antibody or a T-cell antigen receptor (see, e.g., Harlow and Lane (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory; Harlow and Lane (1999) Using antibodies: a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press). FLJ10607 antibodies are further discussed below.

In preferred embodiments, an FLJ10607-interacting protein specifically binds an FLJ10607 protein. In alternative preferred embodiments, an FLJ10607-modulating agent binds an FLJ10607 substrate, binding partner, or cofactor.

Antibodies

In another embodiment, the protein modulator is an FLJ10607 specific antibody agonist or antagonist. The antibodies have therapeutic and diagnostic utilities, and can be used in screening assays to identify FLJ10607 modulators. The antibodies can also be used in dissecting the portions of the FLJ10607 pathway responsible for various cellular responses and in the general processing and maturation of the FLJ10607.

Antibodies that specifically bind FLJ10607 polypeptides can be generated using known methods. Preferably the antibody is specific to a mammalian ortholog of FLJ10607 polypeptide, and more preferably, to human FLJ10607. Antibodies may be polyclonal, monoclonal (mAbs), humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab').sub.2 fragments, fragments produced by a FAb expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. Epitopes of FLJ10607 which are particularly antigenic can be selected, for example, by routine screening of FLJ10607 polypeptides for antigenicity or by

applying a theoretical method for selecting antigenic regions of a protein (Hopp and Wood (1981), Proc. Nati. Acad. Sci. U.S.A. 78:3824-28; Hopp and Wood, (1983) Mol. Immunol. 20:483-89; Sutcliffe et al., (1983) Science 219:660-66) to the amino acid sequence shown in SEQ ID NO:2. Monoclonal antibodies with affinities of 10^8 M^{-1} preferably 10^9 M^{-1} to 10^{10} M^{-1}, or stronger can be made by standard procedures as described (Harlow and Lane, *supra*; Goding (1986) Monoclonal Antibodies: Principles and Practice (2d ed) Academic Press, New York; and U.S. Pat. Nos. 4,381,292; 4,451,570; and 4,618,577). Antibodies may be generated against crude cell extracts of FLJ10607 or substantially purified fragments thereof. If FLJ10607 fragments are used, they preferably comprise at least 10, and more preferably, at least 20 contiguous amino acids of an FLJ10607 protein. In a particular embodiment, FLJ10607-specific antigens and/or immunogens are coupled to carrier proteins that stimulate the immune response. For example, the subject polypeptides are covalently coupled to the keyhole limpet hemocyanin (KLH) carrier, and the conjugate is emulsified in Freund's complete adjuvant, which enhances the immune response. An appropriate immune system such as a laboratory rabbit or mouse is immunized according to conventional protocols.

The presence of FLJ10607-specific antibodies is assayed by an appropriate assay such as a solid phase enzyme-linked immunosorbant assay (ELISA) using immobilized corresponding FLJ10607 polypeptides. Other assays, such as radioimmunoassays or fluorescent assays might also be used.

Chimeric antibodies specific to FLJ10607 polypeptides can be made that contain different portions from different animal species. For instance, a human immunoglobulin constant region may be linked to a variable region of a murine mAb, such that the antibody derives its biological activity from the human antibody, and its binding specificity from the murine fragment. Chimeric antibodies are produced by splicing together genes that encode the appropriate regions from each species (Morrison et al., Proc. Natl. Acad. Sci. (1984) 81:6851-6855; Neuberger et al., Nature (1984) 312:604-608; Takeda et al., Nature (1985) 31:452-454). Humanized antibodies, which are a form of chimeric antibodies, can be generated by grafting complementary-determining regions (CDRs) (Carlos, T. M., J. M. Harlan. 1994. Blood 84:2068-2101) of mouse antibodies into a background of human framework regions and constant regions by recombinant

DNA technology (Riechmann LM, et al., 1988 Nature 323: 323-327). Humanized antibodies contain ~10% murine sequences and ~90% human sequences, and thus further reduce or eliminate immunogenicity, while retaining the antibody specificities (Co MS, and Queen C. 1991 Nature 351: 501-501; Morrison SL. 1992 Ann. Rev. Immun. 10:239-265). Humanized antibodies and methods of their production are well-known in the art (U.S. Pat. Nos. 5,530,101, 5,585,089, 5,693,762, and 6,180,370).

FLJ10607-specific single chain antibodies which are recombinant, single chain polypeptides formed by linking the heavy and light chain fragments of the Fv regions via an amino acid bridge, can be produced by methods known in the art (U.S. Pat. No. 4,946,778; Bird, Science (1988) 242:423-426; Huston et al., Proc. Natl. Acad. Sci. USA (1988) 85:5879-5883; and Ward et al., Nature (1989) 334:544-546).

Other suitable techniques for antibody production involve in vitro exposure of lymphocytes to the antigenic polypeptides or alternatively to selection of libraries of antibodies in phage or similar vectors (Huse et al., Science (1989) 246:1275-1281). As used herein, T-cell antigen receptors are included within the scope of antibody modulators (Harlow and Lane, 1988, *supra*).

The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, antibodies will be labeled by joining, either covalently or non-covalently, a substance that provides for a detectable signal, or that is toxic to cells that express the targeted protein (Menard S, et al., Int J. Biol Markers (1989) 4:131-134). A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, fluorescent emitting lanthanide metals, chemiluminescent moieties, bioluminescent moieties, magnetic particles, and the like (U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241). Also, recombinant immunoglobulins may be produced (U.S. Pat. No. 4,816,567). Antibodies to cytoplasmic polypeptides may be delivered and reach their targets by conjugation with membrane-penetrating toxin proteins (U.S. Pat. No. 6,086,900).

When used therapeutically in a patient, the antibodies of the subject invention are typically administered parenterally, when possible at the target site, or intravenously. The

therapeutically effective dose and dosage regimen is determined by clinical studies. Typically, the amount of antibody administered is in the range of about 0.1 mg/kg –to about 10 mg/kg of patient weight. For parenteral administration, the antibodies are formulated in a unit dosage injectable form (e.g., solution, suspension, emulsion) in association with a pharmaceutically acceptable vehicle. Such vehicles are inherently nontoxic and non-therapeutic. Examples are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Nonaqueous vehicles such as fixed oils, ethyl oleate, or liposome carriers may also be used. The vehicle may contain minor amounts of additives, such as buffers and preservatives, which enhance isotonicity and chemical stability or otherwise enhance therapeutic potential. The antibodies' concentrations in such vehicles are typically in the range of about 1 mg/ml to about10 mg/ml. Immunotherapeutic methods are further described in the literature (US Pat. No. 5,859,206; WO0073469).

Nucleic Acid Modulators

Other preferred FLJ10607-modulating agents comprise nucleic acid molecules, such as antisense oligomers or double stranded RNA (dsRNA), which generally inhibit FLJ10607 activity. Preferred nucleic acid modulators interfere with the function of the FLJ10607 nucleic acid such as DNA replication, transcription, translocation of the FLJ10607 RNA to the site of protein translation, translation of protein from the FLJ10607 RNA, splicing of the FLJ10607 RNA to yield one or more mRNA species, or catalytic activity which may be engaged in or facilitated by the FLJ10607 RNA.

In one embodiment, the antisense oligomer is an oligonucleotide that is sufficiently complementary to an FLJ10607 mRNA to bind to and prevent translation, preferably by binding to the 5′ untranslated region. FLJ10607-specific antisense oligonucleotides, preferably range from at least 6 to about 200 nucleotides. In some embodiments the oligonucleotide is preferably at least 10, 15, or 20 nucleotides in length. In other embodiments, the oligonucleotide is preferably less than 50, 40, or 30 nucleotides in length. The oligonucleotide can be DNA or RNA or a chimeric mixture or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone. The

oligonucleotide may include other appending groups such as peptides, agents that facilitate transport across the cell membrane, hybridization-triggered cleavage agents, and intercalating agents.

In another embodiment, the antisense oligomer is a phosphothioate morpholino oligomer (PMO). PMOs are assembled from four different morpholino subunits, each of which contain one of four genetic bases (A, C, G, or T) linked to a six-membered morpholine ring. Polymers of these subunits are joined by non-ionic phosphodiamidate intersubunit linkages. Details of how to make and use PMOs and other antisense oligomers are well known in the art (e.g. see WO99/18193; Probst JC, Antisense Oligodeoxynucleotide and Ribozyme Design, Methods. (2000) 22(3):271-281; Summerton J, and Weller D. 1997 Antisense Nucleic Acid Drug Dev. :7:187-95; US Pat. No. 5,235,033; and US Pat No. 5,378,841).

Alternative preferred FLJ10607 nucleic acid modulators are double-stranded RNA species mediating RNA interference (RNAi). RNAi is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. Methods relating to the use of RNAi to silence genes in *C. elegans*, *Drosophila*, plants, and humans are known in the art (Fire A, et al., 1998 Nature 391:806-811; Fire, A. Trends Genet. 15, 358-363 (1999); Sharp, P. A. RNA interference 2001. Genes Dev. 15, 485-490 (2001); Hammond, S. M., et al., Nature Rev. Genet. 2, 110-1119 (2001); Tuschl, T. Chem. Biochem. 2, 239-245 (2001); Hamilton, A. et al., Science 286, 950-952 (1999); Hammond, S. M., et al., Nature 404, 293-296 (2000); Zamore, P. D., et al., Cell 101, 25-33 (2000); Bernstein, E., et al., Nature 409, 363-366 (2001); Elbashir, S. M., et al., Genes Dev. 15, 188-200 (2001); WO0129058; WO9932619; Elbashir SM, et al., 2001 Nature 411:494-498).

Nucleic acid modulators are commonly used as research reagents, diagnostics, and therapeutics. For example, antisense oligonucleotides, which are able to inhibit gene expression with exquisite specificity, are often used to elucidate the function of particular genes (see, for example, U.S. Pat. No. 6,165,790). Nucleic acid modulators are also used, for example, to distinguish between functions of various members of a biological pathway. For example, antisense oligomers have been employed as therapeutic moieties

in the treatment of disease states in animals and man and have been demonstrated in numerous clinical trials to be safe and effective (Milligan JF, *et al*, Current Concepts in Antisense Drug Design, J Med Chem. (1993) 36:1923-1937; Tonkinson JL *et al.*, Antisense Oligodeoxynucleotides as Clinical Therapeutic Agents, Cancer Invest. (1996) 14:54-65). Accordingly, in one aspect of the invention, an FLJ10607-specific nucleic acid modulator is used in an assay to further elucidate the role of the FLJ10607 in the axin pathway, and/or its relationship to other members of the pathway. In another aspect of the invention, an FLJ10607-specific antisense oligomer is used as a therapeutic agent for treatment of axin-related disease states.

Assay Systems

The invention provides assay systems and screening methods for identifying specific modulators of FLJ10607 activity. As used herein, an "assay system" encompasses all the components required for performing and analyzing results of an assay that detects and/or measures a particular event. In general, primary assays are used to identify or confirm a modulator's specific biochemical or molecular effect with respect to the FLJ10607 nucleic acid or protein. In general, secondary assays further assess the activity of a FLJ10607 modulating agent identified by a primary assay and may confirm that the modulating agent affects FLJ10607 in a manner relevant to the axin pathway. In some cases, FLJ10607 modulators will be directly tested in a secondary assay.

In a preferred embodiment, the screening method comprises contacting a suitable assay system comprising an FLJ10607 polypeptide or nucleic acid with a candidate agent under conditions whereby, but for the presence of the agent, the system provides a reference activity (e.g. enzymatic activity), which is based on the particular molecular event the screening method detects. A statistically significant difference between the agent-biased activity and the reference activity indicates that the candidate agent modulates FLJ10607 activity, and hence the axin pathway. The FLJ10607 polypeptide or nucleic acid used in the assay may comprise any of the nucleic acids or polypeptides described above.

Primary Assays

The type of modulator tested generally determines the type of primary assay.

Primary assays for small molecule modulators

For small molecule modulators, screening assays are used to identify candidate modulators. Screening assays may be cell-based or may use a cell-free system that recreates or retains the relevant biochemical reaction of the target protein (reviewed in Sittampalam GS *et al.*, Curr Opin Chem Biol (1997) 1:384-91 and accompanying references). As used herein the term "cell-based" refers to assays using live cells, dead cells, or a particular cellular fraction, such as a membrane, endoplasmic reticulum, or mitochondrial fraction. The term "cell free" encompasses assays using substantially purified protein (either endogenous or recombinantly produced), partially purified or crude cellular extracts. Screening assays may detect a variety of molecular events, including protein-DNA interactions, protein-protein interactions (*e.g.*, receptor-ligand binding), transcriptional activity (*e.g.*, using a reporter gene), enzymatic activity (*e.g.*, via a property of the substrate), activity of second messengers, immunogenicty and changes in cellular morphology or other cellular characteristics. Appropriate screening assays may use a wide range of detection methods including fluorescent, radioactive, colorimetric, spectrophotometric, and amperometric methods, to provide a read-out for the particular molecular event detected.

Cell-based screening assays usually require systems for recombinant expression of FLJ10607 and any auxiliary proteins demanded by the particular assay. Appropriate methods for generating recombinant proteins produce sufficient quantities of proteins that retain their relevant biological activities and are of sufficient purity to optimize activity and assure assay reproducibility. Yeast two-hybrid and variant screens, and mass spectrometry provide preferred methods for determining protein-protein interactions and elucidation of protein complexes. In certain applications, when FLJ10607-interacting proteins are used in screens to identify small molecule modulators, the binding specificity of the interacting protein to the FLJ10607 protein may be assayed by various known methods such as substrate processing (e.g. ability of the candidate FLJ10607-specific binding agents to function as negative effectors in FLJ10607-expressing cells), binding equilibrium constants (usually at least about $10^7 M^{-1}$, preferably at least about $10^8 M^{-1}$,

more preferably at least about 10^9 M^{-1}), and immunogenicity (e.g. ability to elicit FLJ10607 specific antibody in a heterologous host such as a mouse, rat, goat or rabbit). For enzymes and receptors, binding may be assayed by, respectively, substrate and ligand processing.

The screening assay may measure a candidate agent's ability to specifically bind to or modulate activity of a FLJ10607 polypeptide, a fusion protein thereof, or to cells or membranes bearing the polypeptide or fusion protein. The FLJ10607 polypeptide can be full length or a fragment thereof that retains functional FLJ10607 activity. The FLJ10607 polypeptide may be fused to another polypeptide, such as a peptide tag for detection or anchoring, or to another tag. The FLJ10607 polypeptide is preferably human FLJ10607, or is an ortholog or derivative thereof as described above. In a preferred embodiment, the screening assay detects candidate agent-based modulation of FLJ10607 interaction with a binding target, such as an endogenous or exogenous protein or other substrate that has FLJ10607 –specific binding activity, and can be used to assess normal FLJ10607 gene function.

Suitable assay formats that may be adapted to screen for FLJ10607 modulators are known in the art. Preferred screening assays are high throughput or ultra high throughput and thus provide automated, cost-effective means of screening compound libraries for lead compounds (Fernandes PB, Curr Opin Chem Biol (1998) 2:597-603; Sundberg SA, Curr Opin Biotechnol 2000, 11:47-53). In one preferred embodiment, screening assays uses fluorescence technologies, including fluorescence polarization, time-resolved fluorescence, and fluorescence resonance energy transfer. These systems offer means to monitor protein-protein or DNA-protein interactions in which the intensity of the signal emitted from dye-labeled molecules depends upon their interactions with partner molecules (*e.g.*, Selvin PR, Nat Struct Biol (2000) 7:730-4; Fernandes PB, *supra*; Hertzberg RP and Pope AJ, Curr Opin Chem Biol (2000) 4:445-451).

A variety of suitable assay systems may be used to identify candidate FLJ10607 and axin pathway modulators (e.g. U.S. Pat. Nos. 5,550,019 and 6,133,437 (apoptosis assays); U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434 (angiogenesis assays), among others). Specific preferred assays are described in more detail below.

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Apoptosis assays. Assays for apoptosis may be performed by terminal deoxynucleotidyl transferase-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay. The TUNEL assay is used to measure nuclear DNA fragmentation characteristic of apoptosis (Lazebnik *et al.*, 1994, Nature 371, 346), by following the incorporation of fluorescein-dUTP (Yonehara *et al.*, 1989, J. Exp. Med. 169, 1747). Apoptosis may further be assayed by acridine orange staining of tissue culture cells (Lucas, R., et al., 1998, Blood 15:4730-41). An apoptosis assay system may comprise a cell that expresses an FLJ10607, and that optionally has defective axin function (e.g. axin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the apoptosis assay system and changes in induction of apoptosis relative to controls where no test agent is added, identify candidate axin modulating agents. In some embodiments of the invention, an apoptosis assay may be used as a secondary assay to test a candidate axin modulating agents that is initially identified using a cell-free assay system. An apoptosis assay may also be used to test whether FLJ10607 function plays a direct role in apoptosis. For example, an apoptosis assay may be performed on cells that over- or under-express FLJ10607 relative to wild type cells. Differences in apoptotic response compared to wild type cells suggests that the FLJ10607 plays a direct role in the apoptotic response. Apoptosis assays are described further in US Pat. No. 6,133,437.

Cell proliferation and cell cycle assays. Cell proliferation may be assayed via bromodeoxyuridine (BRDU) incorporation. This assay identifies a cell population undergoing DNA synthesis by incorporation of BRDU into newly-synthesized DNA. Newly-synthesized DNA may then be detected using an anti-BRDU antibody (Hoshino *et al.*, 1986, Int. J. Cancer 38, 369; Campana *et al.*, 1988, J. Immunol. Meth. 107, 79), or by other means.

Cell proliferation is also assayed via phospho-histone H3 staining, which identifies a cell population undergoing mitosis by phosphorylation of histone H3. Phosphorylation of histone H3 at serine 10 is detected using an antibody specfic to the phosphorylated form of the serine 10 residue of histone H3. (Chadlee,D.N. 1995, J. Biol. Chem 270:20098-105). Cell Proliferation may also be examined using [^{3}H]-thymidine incorporation (Chen, J., 1996, Oncogene 13:1395-403; Jeoung, J., 1995, J. Biol. Chem. 270:18367-73).

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This assay allows for quantitative characterization of S-phase DNA syntheses. In this assay, cells synthesizing DNA will incorporate [^{3}H]-thymidine into newly synthesized DNA. Incorporation can then be measured by standard techniques such as by counting of radioisotope in a scintillation counter (e.g., Beckman LS 3800 Liquid Scintillation Counter). Another proliferation assay uses the dye Alamar Blue (available from Biosource International), which fluoresces when reduced in living cells and provides an indirect measurement of cell number (Voytik-Harbin SL et al., 1998, In Vitro Cell Dev Biol Anim 34:239-46).

Cell proliferation may also be assayed by colony formation in soft agar (Sambrook et al., Molecular Cloning, Cold Spring Harbor (1989)). For example, cells transformed with FLJ10607 are seeded in soft agar plates, and colonies are measured and counted after two weeks incubation.

Involvement of a gene in the cell cycle may be assayed by flow cytometry (Gray JW et al. (1986) Int J Radiat Biol Relat Stud Phys Chem Med 49:237-55). Cells transfected with an FLJ10607 may be stained with propidium iodide and evaluated in a flow cytometer (available from Becton Dickinson), which indicates accumulation of cells in different stages of the cell cycle.

Accordingly, a cell proliferation or cell cycle assay system may comprise a cell that expresses an FLJ10607, and that optionally has defective axin function (e.g. axin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the assay system and changes in cell proliferation or cell cycle relative to controls where no test agent is added, identify candidate axin modulating agents. In some embodiments of the invention, the cell proliferation or cell cycle assay may be used as a secondary assay to test a candidate axin modulating agents that is initially identified using another assay system such as a cell-free assay system. A cell proliferation assay may also be used to test whether FLJ10607 function plays a direct role in cell proliferation or cell cycle. For example, a cell proliferation or cell cycle assay may be performed on cells that over- or under-express FLJ10607 relative to wild type cells. Differences in proliferation or cell cycle compared to wild type cells suggests that the FLJ10607 plays a direct role in cell proliferation or cell cycle.

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Angiogenesis. Angiogenesis may be assayed using various human endothelial cell systems, such as umbilical vein, coronary artery, or dermal cells. Suitable assays include Alamar Blue based assays (available from Biosource International) to measure proliferation; migration assays using fluorescent molecules, such as the use of Becton Dickinson Falcon HTS FluoroBlock cell culture inserts to measure migration of cells through membranes in presence or absence of angiogenesis enhancer or suppressors; and tubule formation assays based on the formation of tubular structures by endothelial cells on Matrigel® (Becton Dickinson). Accordingly, an angiogenesis assay system may comprise a cell that expresses an FLJ10607, and that optionally has defective axin function (e.g. axin is over-expressed or under-expressed relative to wild-type cells). A test agent can be added to the angiogenesis assay system and changes in angiogenesis relative to controls where no test agent is added, identify candidate axin modulating agents. In some embodiments of the invention, the angiogenesis assay may be used as a secondary assay to test a candidate axin modulating agents that is initially identified using another assay system. An angiogenesis assay may also be used to test whether FLJ10607 function plays a direct role in cell proliferation. For example, an angiogenesis assay may be performed on cells that over- or under-express FLJ10607 relative to wild type cells. Differences in angiogenesis compared to wild type cells suggests that the FLJ10607 plays a direct role in angiogenesis. U.S. Pat. Nos. 5,976,782, 6,225,118 and 6,444,434, among others.

Hypoxic induction. The alpha subunit of the transcription factor, hypoxia inducible factor-1 (HIF-1), is upregulated in tumor cells following exposure to hypoxia in vitro. Under hypoxic conditions, HIF-1 stimulates the expression of genes known to be important in tumour cell survival, such as those encoding glyolytic enzymes and VEGF. Induction of such genes by hypoxic conditions may be assayed by growing cells transfected with FLJ10607 in hypoxic conditions (such as with 0.1% O2, 5% CO2, and balance N2, generated in a Napco 7001 incubator (Precision Scientific)) and normoxic conditions, followed by assessment of gene activity or expression by Taqman®. For example, a hypoxic induction assay system may comprise a cell that expresses an FLJ10607, and that optionally has defective axin function (e.g. axin is over-expressed or

under-expressed relative to wild-type cells). A test agent can be added to the hypoxic induction assay system and changes in hypoxic response relative to controls where no test agent is added, identify candidate axin modulating agents. In some embodiments of the invention, the hypoxic induction assay may be used as a secondary assay to test a candidate axin modulating agents that is initially identified using another assay system. A hypoxic induction assay may also be used to test whether FLJ10607 function plays a direct role in the hypoxic response. For example, a hypoxic induction assay may be performed on cells that over- or under-express FLJ10607 relative to wild type cells. Differences in hypoxic response compared to wild type cells suggests that the FLJ10607 plays a direct role in hypoxic induction.

Cell adhesion. Cell adhesion assays measure adhesion of cells to purified adhesion proteins, or adhesion of cells to each other, in presence or absence of candidate modulating agents. Cell-protein adhesion assays measure the ability of agents to modulate the adhesion of cells to purified proteins. For example, recombinant proteins are produced, diluted to 2.5g/mL in PBS, and used to coat the wells of a microtiter plate. The wells used for negative control are not coated. Coated wells are then washed, blocked with 1% BSA, and washed again. Compounds are diluted to 2× final test concentration and added to the blocked, coated wells. Cells are then added to the wells, and the unbound cells are washed off. Retained cells are labeled directly on the plate by adding a membrane-permeable fluorescent dye, such as calcein-AM, and the signal is quantified in a fluorescent microplate reader.

Cell-cell adhesion assays measure the ability of agents to modulate binding of cell adhesion proteins with their native ligands. These assays use cells that naturally or recombinantly express the adhesion protein of choice. In an exemplary assay, cells expressing the cell adhesion protein are plated in wells of a multiwell plate. Cells expressing the ligand are labeled with a membrane-permeable fluorescent dye, such as BCECF , and allowed to adhere to the monolayers in the presence of candidate agents. Unbound cells are washed off, and bound cells are detected using a fluorescence plate reader.

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High-throughput cell adhesion assays have also been described. In one such assay, small molecule ligands and peptides are bound to the surface of microscope slides using a microarray spotter, intact cells are then contacted with the slides, and unbound cells are washed off. In this assay, not only the binding specificity of the peptides and modulators against cell lines are determined, but also the functional cell signaling of attached cells using immunofluorescence techniques in situ on the microchip is measured (Falsey JR et al., Bioconjug Chem. 2001 May-Jun;12(3):346-53).

Tubulogenesis. Tubulogenesis assays monitor the ability of cultured cells, generally endothelial cells, to form tubular structures on a matrix substrate, which generally simulates the environment of the extracellular matrix. Exemplary substrates include Matrigel™ (Becton Dickinson), an extract of basement membrane proteins containing laminin, collagen IV, and heparin sulfate proteoglycan, which is liquid at 4° C and forms a solid gel at 37° C. Other suitable matrices comprise extracellular components such as collagen, fibronectin, and/or fibrin. Cells are stimulated with a pro-angiogenic stimulant, and their ability to form tubules is detected by imaging. Tubules can generally be detected after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Tube formation assays are well known in the art (e.g., Jones MK et al., 1999, Nature Medicine 5:1418-1423). These assays have traditionally involved stimulation with serum or with the growth factors FGF or VEGF. Serum represents an undefined source of growth factors. In a preferred embodiment, the assay is performed with cells cultured in serum free medium, in order to control which process or pathway a candidate agent modulates. Moreover, we have found that different target genes respond differently to stimulation with different pro-angiogenic agents, including inflammatory angiogenic factors such as TNF-alpa. Thus, in a further preferred embodiment, a tubulogenesis assay system comprises testing an FLJ10607's response to a variety of factors, such as FGF, VEGF, phorbol myristate acetate (PMA), TNF-alpha, ephrin, etc.

Cell Migration. An invasion/migration assay (also called a migration assay) tests the ability of cells to overcome a physical barrier and to migrate towards pro-angiogenic

signals. Migration assays are known in the art (e.g., Paik JH et al., 2001, J Biol Chem 276:11830-11837). In a typical experimental set-up, cultured endothelial cells are seeded onto a matrix-coated porous lamina, with pore sizes generally smaller than typical cell size. The matrix generally simulates the environment of the extracellular matrix, as described above. The lamina is typically a membrane, such as the transwell polycarbonate membrane (Corning Costar Corporation, Cambridge, MA), and is generally part of an upper chamber that is in fluid contact with a lower chamber containing pro-angiogenic stimuli. Migration is generally assayed after an overnight incubation with stimuli, but longer or shorter time frames may also be used. Migration is assessed as the number of cells that crossed the lamina, and may be detected by staining cells with hemotoxylin solution (VWR Scientific, South San Francisco, CA), or by any other method for determining cell number. In another exemplary set up, cells are fluorescently labeled and migration is detected using fluorescent readings, for instance using the Falcon HTS FluoroBlok (Becton Dickinson). While some migration is observed in the absence of stimulus, migration is greatly increased in response to pro-angiogenic factors. As described above, a preferred assay system for migration/invasion assays comprises testing an FLJ10607's response to a variety of pro-angiogenic factors, including tumor angiogenic and inflammatory angiogenic agents, and culturing the cells in serum free medium.

Sprouting assay. A sprouting assay is a three-dimensional *in vitro* angiogenesis assay that uses a cell-number defined spheroid aggregation of endothelial cells ("spheroid"), embedded in a collagen gel-based matrix. The spheroid can serve as a starting point for the sprouting of capillary-like structures by invasion into the extracellular matrix (termed "cell sprouting") and the subsequent formation of complex anastomosing networks (Korff and Augustin, 1999, J Cell Sci 112:3249-58). In an exemplary experimental set-up, spheroids are prepared by pipetting 400 human umbilical vein endothelial cells into individual wells of a nonadhesive 96-well plates to allow overnight spheroidal aggregation (Korff and Augustin: J Cell Biol 143: 1341-52, 1998). Spheroids are harvested and seeded in 900μl of methocel-collagen solution and pipetted into individual wells of a 24 well plate to allow collagen gel polymerization. Test agents

are added after 30 min by pipetting 100 µl of 10-fold concentrated working dilution of the test substances on top of the gel. Plates are incubated at 37°C for 24h. Dishes are fixed at the end of the experimental incubation period by addition of paraformaldehyde. Sprouting intensity of endothelial cells can be quantitated by an automated image analysis system to determine the cumulative sprout length per spheroid.

Primary assays for antibody modulators

For antibody modulators, appropriate primary assays test is a binding assay that tests the antibody's affinity to and specificity for the FLJ10607 protein. Methods for testing antibody affinity and specificity are well known in the art (Harlow and Lane, 1988, 1999, *supra*). The enzyme-linked immunosorbant assay (ELISA) is a preferred method for detecting FLJ10607-specific antibodies; others include FACS assays, radioimmunoassays, and fluorescent assays.

In some cases, screening assays described for small molecule modulators may also be used to test antibody modulators.

Primary assays for nucleic acid modulators

For nucleic acid modulators, primary assays may test the ability of the nucleic acid modulator to inhibit or enhance FLJ10607 gene expression, preferably mRNA expression. In general, expression analysis comprises comparing FLJ10607 expression in like populations of cells (*e.g.*, two pools of cells that endogenously or recombinantly express FLJ10607) in the presence and absence of the nucleic acid modulator. Methods for analyzing mRNA and protein expression are well known in the art. For instance, Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR (*e.g.*, using the TaqMan®, PE Applied Biosystems), or microarray analysis may be used to confirm that FLJ10607 mRNA expression is reduced in cells treated with the nucleic acid modulator (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm DH and Guiseppi-Elie, A Curr Opin Biotechnol 2001, 12:41-47). Protein expression may also be monitored. Proteins are most commonly detected with specific antibodies or antisera directed against either

the FLJ10607 protein or specific peptides. A variety of means including Western blotting, ELISA, or in situ detection, are available (Harlow E and Lane D, 1988 and 1999, *supra*).

In some cases, screening assays described for small molecule modulators, particularly in assay systems that involve FLJ10607 mRNA expression, may also be used to test nucleic acid modulators.

Secondary Assays

Secondary assays may be used to further assess the activity of FLJ10607-modulating agent identified by any of the above methods to confirm that the modulating agent affects FLJ10607 in a manner relevant to the axin pathway. As used herein, FLJ10607-modulating agents encompass candidate clinical compounds or other agents derived from previously identified modulating agent. Secondary assays can also be used to test the activity of a modulating agent on a particular genetic or biochemical pathway or to test the specificity of the modulating agent's interaction with FLJ10607.

Secondary assays generally compare like populations of cells or animals (*e.g.*, two pools of cells or animals that endogenously or recombinantly express FLJ10607) in the presence and absence of the candidate modulator. In general, such assays test whether treatment of cells or animals with a candidate FLJ10607–modulating agent results in changes in the axin pathway in comparison to untreated (or mock- or placebo-treated) cells or animals. Certain assays use "sensitized genetic backgrounds", which, as used herein, describe cells or animals engineered for altered expression of genes in the axin or interacting pathways.

Cell-based assays

Cell based assays may detect endogenous axin pathway activity or may rely on recombinant expression of axin pathway components. Any of the aforementioned assays may be used in this cell-based format. Candidate modulators are typically added to the cell media but may also be injected into cells or delivered by any other efficacious means.

Animal Assays

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A variety of non-human animal models of normal or defective axin pathway may be used to test candidate FLJ10607 modulators. Models for defective axin pathway typically use genetically modified animals that have been engineered to mis-express (*e.g.*, over-express or lack expression in) genes involved in the axin pathway. Assays generally require systemic delivery of the candidate modulators, such as by oral administration, injection, etc.

In a preferred embodiment, axin pathway activity is assessed by monitoring neovascularization and angiogenesis. Animal models with defective and normal axin are used to test the candidate modulator's affect on FLJ10607 in Matrigel® assays. Matrigel® is an extract of basement membrane proteins, and is composed primarily of laminin, collagen IV, and heparin sulfate proteoglycan. It is provided as a sterile liquid at 4°C, but rapidly forms a solid gel at 37°C. Liquid Matrigel® is mixed with various angiogenic agents, such as bFGF and VEGF, or with human tumor cells which over-express the FLJ10607. The mixture is then injected subcutaneously(SC) into female athymic nude mice (Taconic, Germantown, NY) to support an intense vascular response. Mice with Matrigel® pellets may be dosed via oral (PO), intraperitoneal (IP), or intravenous (IV) routes with the candidate modulator. Mice are euthanized 5 - 12 days post-injection, and the Matrigel® pellet is harvested for hemoglobin analysis (Sigma plasma hemoglobin kit). Hemoglobin content of the gel is found to correlate the degree of neovascularization in the gel.

In another preferred embodiment, the effect of the candidate modulator on FLJ10607 is assessed via tumorigenicity assays. Tumor xenograft assays are known in the art (see, e.g., Ogawa K et al., 2000, Oncogene 19:6043-6052). Xenografts are typically implanted SC into female athymic mice, 6-7 week old, as single cell suspensions either from a pre-existing tumor or from *in vitro* culture. The tumors which express the FLJ10607 endogenously are injected in the flank, 1 x 10^5 to 1 x 10^7 cells per mouse in a volume of 100 µL using a 27gauge needle. Mice are then ear tagged and tumors are measured twice weekly. Candidate modulator treatment is initiated on the day the mean tumor weight reaches 100 mg. Candidate modulator is delivered IV, SC, IP, or PO by bolus administration. Depending upon the pharmacokinetics of each unique candidate modulator, dosing can be performed multiple times per day. The tumor weight is

assessed by measuring perpendicular diameters with a caliper and calculated by multiplying the measurements of diameters in two dimensions. At the end of the experiment, the excised tumors maybe utilized for biomarker identification or further analyses. For immunohistochemistry staining, xenograft tumors are fixed in 4% paraformaldehyde, 0.1M phosphate, pH 7.2, for 6 hours at 4°C, immersed in 30% sucrose in PBS, and rapidly frozen in isopentane cooled with liquid nitrogen.

In another preferred embodiment, tumorogenicity is monitored using a hollow fiber assay, which is described in U.S. Pat No. US 5,698,413. Briefly, the method comprises implanting into a laboratory animal a biocompatible, semi-permeable encapsulation device containing target cells, treating the laboratory animal with a candidate modulating agent, and evaluating the target cells for reaction to the candidate modulator. Implanted cells are generally human cells from a pre-existing tumor or a tumor cell line. After an appropriate period of time, generally around six days, the implanted samples are harvested for evaluation of the candidate modulator. Tumorogenicity and modulator efficacy may be evaluated by assaying the quantity of viable cells present in the macrocapsule, which can be determined by tests known in the art, for example, MTT dye conversion assay, neutral red dye uptake, trypan blue staining, viable cell counts, the number of colonies formed in soft agar, the capacity of the cells to recover and replicate in vitro, etc.

In another preferred embodiment, a tumorogenicity assay use a transgenic animal, usually a mouse, carrying a dominant oncogene or tumor suppressor gene knockout under the control of tissue specific regulatory sequences; these assays are generally referred to as transgenic tumor assays. In a preferred application, tumor development in the transgenic model is well characterized or is controlled. In an exemplary model, the "RIP1-Tag2" transgene, comprising the SV40 large T-antigen oncogene under control of the insulin gene regulatory regions is expressed in pancreatic beta cells and results in islet cell carcinomas (Hanahan D, 1985, Nature 315:115-122; Parangi S et al, 1996, Proc Natl Acad Sci USA 93: 2002-2007; Bergers G et al, 1999, Science 284:808-812). An "angiogenic switch," occurs at approximately five weeks, as normally quiescent capillaries in a subset of hyperproliferative islets become angiogenic. The RIP1-TAG2 mice die by age 14 weeks. Candidate modulators may be administered

at a variety of stages, including just prior to the angiogenic switch (e.g., for a model of tumor prevention), during the growth of small tumors (e.g., for a model of intervention), or during the growth of large and/or invasive tumors (e.g., for a model of regression). Tumorogenicity and modulator efficacy can be evaluating life-span extension and/or tumor characteristics, including number of tumors, tumor size, tumor morphology, vessel density, apoptotic index, etc.

Diagnostic and therapeutic uses

Specific FLJ10607-modulating agents are useful in a variety of diagnostic and therapeutic applications where disease or disease prognosis is related to defects in the axin pathway, such as angiogenic, apoptotic, or cell proliferation disorders. Accordingly, the invention also provides methods for modulating the axin pathway in a cell, preferably a cell pre-determined to have defective or impaired axin function (e.g. due to overexpression, underexpression, or misexpression of axin, or due to gene mutations), comprising the step of administering an agent to the cell that specifically modulates FLJ10607 activity. Preferably, the modulating agent produces a detectable phenotypic change in the cell indicating that the axin function is restored. The phrase "function is restored", and equivalents, as used herein, means that the desired phenotype is achieved, or is brought closer to normal compared to untreated cells. For example, with restored axin function, cell proliferation and/or progression through cell cycle may normalize, or be brought closer to normal relative to untreated cells. The invention also provides methods for treating disorders or disease associated with impaired axin function by administering a therapeutically effective amount of an FLJ10607 -modulating agent that modulates the axin pathway. The invention further provides methods for modulating FLJ10607 function in a cell, preferably a cell pre-determined to have defective or impaired FLJ10607 function, by administering an FLJ10607 -modulating agent. Additionally, the invention provides a method for treating disorders or disease associated with impaired FLJ10607 function by administering a therapeutically effective amount of an FLJ10607 -modulating agent.

The discovery that FLJ10607 is implicated in axin pathway provides for a variety of methods that can be employed for the diagnostic and prognostic evaluation of diseases

and disorders involving defects in the axin pathway and for the identification of subjects having a predisposition to such diseases and disorders.

Various expression analysis methods can be used to diagnose whether FLJ10607 expression occurs in a particular sample, including Northern blotting, slot blotting, ribonuclease protection, quantitative RT-PCR, and microarray analysis. (*e.g.*, Current Protocols in Molecular Biology (1994) Ausubel FM *et al.*, *eds.*, John Wiley & Sons, Inc., chapter 4; Freeman WM *et al.*, Biotechniques (1999) 26:112-125; Kallioniemi OP, Ann Med 2001, 33:142-147; Blohm and Guiseppi-Elie, Curr Opin Biotechnol 2001, 12:41-47). Tissues having a disease or disorder implicating defective axin signaling that express an FLJ10607, are identified as amenable to treatment with an FLJ10607 modulating agent. In a preferred application, the axin defective tissue overexpresses an FLJ10607 relative to normal tissue. For example, a Northern blot analysis of mRNA from tumor and normal cell lines, or from tumor and matching normal tissue samples from the same patient, using full or partial FLJ10607 cDNA sequences as probes, can determine whether particular tumors express or overexpress FLJ10607. Alternatively, the TaqMan® is used for quantitative RT-PCR analysis of FLJ10607 expression in cell lines, normal tissues and tumor samples (PE Applied Biosystems).

Various other diagnostic methods may be performed, for example, utilizing reagents such as the FLJ10607 oligonucleotides, and antibodies directed against an FLJ10607, as described above for: (1) the detection of the presence of FLJ10607 gene mutations, or the detection of either over- or under-expression of FLJ10607 mRNA relative to the non-disorder state; (2) the detection of either an over- or an under-abundance of FLJ10607 gene product relative to the non-disorder state; and (3) the detection of perturbations or abnormalities in the signal transduction pathway mediated by FLJ10607.

Thus, in a specific embodiment, the invention is drawn to a method for diagnosing a disease or disorder in a patient that is associated with alterations in FLJ10607 expression, the method comprising: a) obtaining a biological sample from the patient; b) contacting the sample with a probe for FLJ10607 expression; c) comparing results from step (b) with a control; and d) determining whether step (c) indicates a likelihood of the disease or disorder. Preferably, the disease is cancer, most preferably a cancer as shown in TABLE 1?. The probe may be either DNA or protein, including an antibody.

EXAMPLES

The following experimental section and examples are offered by way of illustration and not by way of limitation.

I. C. elegans axin screen

We have found that the temperature-sensitive, reduction-of-function *pry-1* mutant *mu38* grown at 15°C produces a ruptured vulva (Rvl) phenotype by which about 95% of animals become eviscerated and die at the L4 molt. The *pry-1* Rvl mutant phenotype is suppressed by loss-of-function mutations in the beta-catenin ortholog *bar-1* and the TCF ortholog *pop-1*. The Rvl phenotype can also be generated by gain-of-function mutations in *bar-1*/beta-catenin that eliminate the consensus GSK3-beta phosphorylation sites and are predicted to prevent Axin-mediated degradation of BAR-1.

We designed a genetic screen to identify genes in addition to *bar-1*/beta-catenin and *pop-1*/TCF that act positively in beta-catenin signaling and, when inactivated, suppress the Rvl mutant phenotype of *pry-1*/Axin. The function of individual genes was inactivated by RNAi in *pry-1 (mu38)* L1 larvae, and suppression of the Rvl phenotype was scored as a statistically significant increase in the proportion of larvae that survived to adulthood without rupturing. Suppressor genes were subsequently counterscreened to eliminate those that appeared to suppress the *pry-1* mutant non-specifically, rather than those that specifically functioned in beta-catenin signaling. Suppressor genes that did not block vulva formation in a wildtype background, and that did not suppress the Rvl phenotype of two mutations in genes unrelated to beta-catenin signaling (*lin-1*/Ets and *daf-18*/PTEN) were considered to be specific *pry-1*/Axin suppressors. These suppressor genes, when inactivated, likely suppress beta-catenin's inappropriate transcriptional activation of target genes and, therefore, may be relevant for cancer therapy.

II. High-Throughput In Vitro Fluorescence Polarization Assay

Fluorescently-labeled FLJ10607 peptide/substrate are added to each well of a 96-well microtiter plate, along with a test agent in a test buffer (10 mM HEPES, 10 mM NaCl, 6 mM magnesium chloride, pH 7.6). Changes in fluorescence polarization,

determined by using a Fluorolite FPM-2 Fluorescence Polarization Microtiter System (Dynatech Laboratories, Inc), relative to control values indicates the test compound is a candidate modifier of FLJ10607 activity.

III. High-Throughput In Vitro Binding Assay.

^{33}P-labeled FLJ10607 peptide is added in an assay buffer (100 mM KCl, 20 mM HEPES pH 7.6, 1 mM $MgCl_2$, 1% glycerol, 0.5% NP-40, 50 mM beta-mercaptoethanol, 1 mg/ml BSA, cocktail of protease inhibitors) along with a test agent to the wells of a Neutralite-avidin coated assay plate and incubated at 25°C for 1 hour. Biotinylated substrate is then added to each well and incubated for 1 hour. Reactions are stopped by washing with PBS, and counted in a scintillation counter. Test agents that cause a difference in activity relative to control without test agent are identified as candidate axin modulating agents.

IV. Immunoprecipitations and Immunoblotting

For coprecipitation of transfected proteins, 3×10^6 appropriate recombinant cells containing the FLJ10607 proteins are plated on 10-cm dishes and transfected on the following day with expression constructs. The total amount of DNA is kept constant in each transfection by adding empty vector. After 24 h, cells are collected, washed once with phosphate-buffered saline and lysed for 20 min on ice in 1 ml of lysis buffer containing 50 mM Hepes, pH 7.9, 250 mM NaCl, 20 mM -glycerophosphate, 1 mM sodium orthovanadate, 5 mM p-nitrophenyl phosphate, 2 mM dithiothreitol, protease inhibitors (complete, Roche Molecular Biochemicals), and 1% Nonidet P-40. Cellular debris is removed by centrifugation twice at 15,000 × g for 15 min. The cell lysate is incubated with 25 μl of M2 beads (Sigma) for 2 h at 4 °C with gentle rocking.

After extensive washing with lysis buffer, proteins bound to the beads are solubilized by boiling in SDS sample buffer, fractionated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane and blotted with the indicated antibodies. The reactive bands are visualized with horseradish peroxidase coupled to the appropriate secondary antibodies and the enhanced chemiluminescence (ECL) Western blotting detection system (Amersham Pharmacia Biotech).

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V. Expression analysis

All cell lines used in the following experiments are NCI (National Cancer Institute) lines, and are available from ATCC (American Type Culture Collection, Manassas, VA 20110-2209). Normal and tumor tissues are obtained from Impath, UC Davis, Clontech, Stratagene, Ardais, Genome Collaborative, and Ambion.

TaqMan analysis is used to assess expression levels of the disclosed genes in various samples.

RNA is extracted from each tissue sample using Qiagen (Valencia, CA) RNeasy kits, following manufacturer's protocols, to a final concentration of 50ng/μl. Single stranded cDNA is then synthesized by reverse transcribing the RNA samples using random hexamers and 500ng of total RNA per reaction, following protocol 4304965 of Applied Biosystems (Foster City, CA).

Primers for expression analysis using TaqMan assay (Applied Biosystems, Foster City, CA) are prepared according to the TaqMan protocols, and the following criteria: a) primer pairs are designed to span introns to eliminate genomic contamination, and b) each primer pair produced only one product. Expression analysis is performed using a 7900HT instrument.

Taqman reactions are carried out following manufacturer's protocols, in 25 μl total volume for 96-well plates and 10 μl total volume for 384-well plates, using 300nM primer and 250 nM probe, and approximately 25ng of cDNA. The standard curve for result analysis is prepared using a universal pool of human cDNA samples, which is a mixture of cDNAs from a wide variety of tissues so that the chance that a target will be present in appreciable amounts is good. The raw data are normalized using 18S rRNA (universally expressed in all tissues and cells).

For each expression analysis, tumor tissue samples are compared with matched normal tissues from the same patient. A gene is considered overexpressed in a tumor when the level of expression of the gene is 2 fold or higher in the tumor compared with its matched normal sample. In cases where normal tissue is not available, a universal pool of cDNA samples is used instead. In these cases, a gene is considered overexpressed in a tumor sample when the difference of expression levels between a

tumor sample and the average of all normal samples from the same tissue type is greater than 2 times the standard deviation of all normal samples (i.e., Tumor – average(all normal samples) > 2 x STDEV(all normal samples)).

A modulator identified by an assay described herein can be further validated for therapeutic effect by administration to a tumor in which the gene is overexpressed. A decrease in tumor growth confirms therapeutic utility of the modulator. Prior to treating a patient with the modulator, the likelihood that the patient will respond to treatment can be diagnosed by obtaining a tumor sample from the patient, and assaying for expression of the gene targeted by the modulator. The expression data for the gene(s) can also be used as a diagnostic marker for disease progression. The assay can be performed by expression analysis as described above, by antibody directed to the gene target, or by any other available detection method.

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WHAT IS CLAIMED IS:

1. A method of identifying a candidate axin pathway modulating agent, said method comprising the steps of:

(a) providing an assay system comprising a FLJ10607 polypeptide or nucleic acid;

(b) contacting the assay system with a test agent under conditions whereby, but for the presence of the test agent, the system provides a reference activity; and

(c) detecting a test agent-biased activity of the assay system, wherein a difference between the test agent-biased activity and the reference activity identifies the test agent as a candidate axin pathway modulating agent.

2. The method of Claim 1 wherein the assay system comprises cultured cells that express the FLJ10607 polypeptide.

3. The method of Claim 2 wherein the cultured cells additionally have defective axin function.

4. The method of Claim 1 wherein the assay system includes a screening assay comprising a FLJ10607 polypeptide, and the candidate test agent is a small molecule modulator.

5. The method of Claim 4 wherein the assay is an enzymatic assay.

6. The method of Claim 1 wherein the assay system is selected from the group consisting of an apoptosis assay system, a cell proliferation assay system, an angiogenesis assay system, and a hypoxic induction assay system.

7. The method of Claim 1 wherein the assay system includes a binding assay comprising a FLJ10607 polypeptide and the candidate test agent is an antibody.

8. The method of Claim 1 wherein the assay system includes an expression assay comprising a FLJ10607 nucleic acid and the candidate test agent is a nucleic acid modulator.

9. The method of claim 8 wherein the nucleic acid modulator is an antisense oligomer.

10. The method of Claim 8 wherein the nucleic acid modulator is a PMO.

11. The method of Claim 1 additionally comprising:

(d) administering the candidate axin pathway modulating agent identified in (c) to a model system comprising cells defective in axin function and, detecting a phenotypic change in the model system that indicates that the axin function is restored.

12. The method of Claim 11 wherein the model system is a mouse model with defective axin function.

13. A method for modulating a axin pathway of a cell comprising contacting a cell defective in axin function with a candidate modulator that specifically binds to a FLJ10607 polypeptide, whereby axin function is restored.

14. The method of claim 13 wherein the candidate modulator is administered to a vertebrate animal predetermined to have a disease or disorder resulting from a defect in axin function.

15. The method of Claim 13 wherein the candidate modulator is selected from the group consisting of an antibody and a small molecule.

16. The method of Claim 1, comprising the additional steps of:

(d) providing a secondary assay system comprising cultured cells or a non-human animal expressing FLJ10607 ,

(e) contacting the secondary assay system with the test agent of (b) or an agent derived therefrom under conditions whereby, but for the presence of the test agent or agent derived therefrom, the system provides a reference activity; and

(f) detecting an agent-biased activity of the second assay system,

wherein a difference between the agent-biased activity and the reference activity of the second assay system confirms the test agent or agent derived therefrom as a candidate axin pathway modulating agent,

and wherein the second assay detects an agent-biased change in the axin pathway.

17. The method of Claim 16 wherein the secondary assay system comprises cultured cells.

18. The method of Claim 16 wherein the secondary assay system comprises a non-human animal.

19. The method of Claim 18 wherein the non-human animal mis-expresses a axin pathway gene.

20. A method of modulating axin pathway in a mammalian cell comprising contacting the cell with an agent that specifically binds a FLJ10607 polypeptide or nucleic acid.

21. The method of Claim 20 wherein the agent is administered to a mammalian animal predetermined to have a pathology associated with the axin pathway.

22. The method of Claim 20 wherein the agent is a small molecule modulator, a nucleic acid modulator, or an antibody.

23. A method for diagnosing a disease in a patient comprising:

(a) obtaining a biological sample from the patient;

(b) contacting the sample with a probe for FLJ10607 expression;

(c) comparing results from step (b) with a control;

(d) determining whether step (c) indicates a likelihood of disease.

24. The method of claim 23 wherein said disease is cancer.

ABSTRACT OF THE DISCLOSURE

Human FLJ10607 genes are identified as modulators of the axin pathway, and thus are therapeutic targets for disorders associated with defective axin function. Methods for identifying modulators of axin, comprising screening for agents that modulate the activity of FLJ10607 are provided.

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Nucleic Acid and Polypeptide sequences

SEQ ID NO:1

>gi|18597005|ref|XM_085119.1| Homo sapiens hypothetical protein FLJ10607 similar to glucosamine-phosphate N-acetyltransferase (FLJ10607), mRNA

GGGCGGGTGGCGCCTTGGCCTCCGCCTCCGCTCGCCTGCGCGCGGCCCTGCGTGAGGGGGCAGAGGCGAG
GTGGAGGCGTTGGCGCTGCCACGTCTGGGCCGCGGTTCCCAACTGTGGCGCGGGCGGTGGAGGAGGAGGT
GGGGCTGGCGCTGAAGCCGGATCCGGATCCGGTGCTGTGCACACTGGTGGGGGAGAGTCCGACGCGCCTG
GCTAGGAGCGCCGACCGCAGGGCCTCTACGGTTCCTGTAACCAGCACAGTGCCTGATTCATGAATTAAAG
ACCTTACTAGAAAAATGAAACCTGATGAAACTCCTATGTTTGACCCAAGTCTACTCAAAGAAGTGGACTG
GAGTCAGAATACAGCTACATTTTCTCCAGCCATTTCCCCAACACATCCTGGAGAAGGCTTGGTTTTGAGG
CCTCTTTGTACTGCTGACTTAAATAGAGGTTTTTTTAAGGTATTGGGTCAGCTAACAGAGACTGGAGTTG
TCAGCCCTGAACAATTTATGAAATCTTTTGAGCATATGAAGAAATCTGGGGATTATTATGTTACAGTTGT
AGAAGATGTGACTCTAGGACAGATTGTTGCTACGGCAACTCTGATTATAGAACATAAATTCATCCATTCC
TGTGCTAAGAGAGGAAGAGTAGAAGATGTTGTTGTTAGTGATGAATGCAGAGGAAAGCAGCTTGGCAAAT
TGTTATTATCAACCCTTACTTTGCTAAGCAAGAAACTGAACTGTTACAAGATTACCCTTGAATGTCTACC
ACAAAATGTTGGTTTCTATAAAAAGTTTGGATATACTGTATCTGAAGAAAACTACATGTGTCGGAGGTTT
CTAAAGTAAAAATCTTGTAAGAAAATTGTCAAAGGGGCTAATGCTACAAGGCTACACTCTTCCTAGAGTT
GAAATATTTTGTTGCTGCAGCCGAGTGACCTCCATAAATACTGGACTGAAAAAACATTGTAATACTACAA
GTATAATGACATTTAGAAGATTACTTTGGGCTGGTGGGACATGCTGTGAATTTAGATTACAAATGAATAT
TATAAAGGGGATGATTTTTAACCAAAGGAATATATTTTTAACTTGAATCTTTTCTTGCATTGTATTTTTC
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TTGCTCATTTAAAAAAAGTATACATTGAATAAGGCTGTTTATCACATGCATAAAATTAAATATTTTTGTT
TCAAAGAAACATCTCAATACACTTAGGGGTGTATTGTTTCCCACATATTAAGTCAGGGTGGATAAATTAG
TTATTATAACTAAACATAGTATAGTCCAACATTCGTTGATCCCAATACAGGCAAACAACCTGGTCAACCT
TTTGAAGTAGAAGAAATGAAAATTACTTGACAAGATTAAAAGTAAAACAATTTAAATGTTTTACTGAAAG
TTTATATAGTATAGTCTATGTAGATAAAAAGTACCACTTGTCTTTTCTGTGAATTATGACTATTCATTTG
TTAAAAATACCTAAGAGCAATTATAGTGGGACATCTAAGGTCCTCTGTAAACAGTGAATTAGCAAACCTC
AGCCTATGTGTTTCTACCCTGATTTTTTTCTTTTCATGGGTATCTGAAGCCTCTAAGTTTTTTCAAAAAT
GGAGTATCACAAAATTGAGTGAAACACAATACTTAATGTATTGTACTAGATTGCCAAATTCATAAAATGT
TAATGGAAGCTTTTTGATGTGATTATAATGGCACTATTCTGGTCATTATCCTATTTTGATTTTATTTAAT
TTTTTAAAGTTGAAGAATTAAATATTTTAATGGTTCTAATCTTTTGCATTCCATGTTGCATTAAACCTGT
TTATATGAGTAGTCTTCTGTTAGAATCACATCTGTGCTTTTCTTGAGTCTGCTGTTGAACTATTAGATTA
AGTCATAATTCATAAAATTTTAGTTTAATGTGCTCTTTGTAAAATGAAATTGTAAAGAAAATACCAGTGT
TTCTCATCCCATTGACTCACACCACGTCATCTGGATTTTGGATTTCCCTCCATGCAGCCAGCTATAGTTG
GCTTTCCAAAACAACAGAAATCCTTCACCAATAGAGTGCACTACTTACCTGCTTATAGCCTATACAGACG
AACTGATCTGTCCTTCGTGAAACGCAACAAAGCTAGTTCTGTCTTTTCAGAAGTCCTACAACCTTGACAA
AGAGTAGTTTTATCAGGTAAATCCTGGTAATTAAAAACGCATGTTTTTAAAAATTAGCCTGGTAAGGCCG
GGTGCAGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCTGAGGTGGGCAGATCACAAGGTCAGGAG
TTTGAGACCAGCCTGACCAAAATGGTGAAACCCTGTCTCTACTAAAAAAAAGAAAAATTAGCCAGACGTG
GTGGCATGCGCCTGTAATCCCAGCTACTCAGGAAGCTGAGGCGAGAGAATCGCTTGAACCCGGGAGGCAA
AGGTTGCAGTGAGCTGAGATCACACCACTGCACTCCAGCCTGGCGACAGAGAGAGACTCCATCTCAAAAC
AAAACAAAAAAAATTAGCCTACTTAAAGGCACAACTAAATGCTTTATTACCTTTCTTACCACTGAACAAT
TTGAGGTAAAATCATTCACAAGGTTGGCACTTCAGTAAATCCCTTTAAATAGTGTTCCTAAGATATCTCT
TAAATCCTCCCATAGGAAATAGAATTACAGGTAAGGTACACCATACAAAAATTGTGTCATTGAGGACAAT
GGTGATCTGTAATTTTAGTTGAGTATGTTTATGATTTTTGAAGCCATATGGTGAGTAAATGTAAATATGA
AAAAAGTGCTACATAAAACACTTCTTAAACTTTTTTTTTTTAAAAACTGCTCCTTGTGGAGCAGGACTAC
CCCATAGGCAGTGTACCCACAATAGATAGCCTTTTGTTGTTGTTGTTGTTGAGACAAGGTCTCGCTGTTG
CCCAGGCTAGAGCGCAGGGGCACAATCACCACACACTGCCGCTTCAATCTCCTGGGCTCAAATGATCCTT
CCACCTCAGCCTCCCATGTGGCTGGGACTATAGGTGCATGCCACCACACCCAGCTAATTAAAAAAATTTT
TTGTGTGGAGTCTATGTTGCCCAGGCTGGTCTCTTAACTCCTAGGCTCAAGTGATCCTCCCACCTCAGCC
TCCCAAAGTGCTTGGATGACAGGTGCGAGCCACTGCACCTGGCCCACATTTTTTAAAGAGACACTGTCCC
ACTCCATCACCCAGGCTGGAGTCCAGTGGTGTGATCATAGCTCACTGCATCCTCCAGTTCCTGGGTTCAA
GCCATCCCTCCTGCCTCAGCCTCCCCAGTAGCTGGAACTACAGGTGTGTGCCATCACACCTGGCTTTACA
TTTTTCTGTGGGGTCTTACTATATTGCCCAAGCCGGTCTCAAACTCCTGAGCTCAAGTGATCCTCTGCCT

EX02-147P

CAGCCTCCAGAGTATCTGGGATTACATATGTCGGCTACCGTGTCTGGCCGTTCACATCTTTGGCCACTAT
TTGCTTGTGAAAAGGTATAATGAGGTGGTACTTATCATTTTTACTGTGTCTCATGTTTTGTATATTTTTG
TTTCATCAACTAAGATGCACTGTAACATCTCTGAAATCTGGATATATTATCAATGGTTTATCATAGTTTT
GTTAGCAATACACTGTCTTTTAGTGGTGCCTAAAATAATGGTATAGTTGTGAGGTGATCTTAGATTTGAT
GAAGCACAGTATGCAGGTAGGCCTAATGGGGGAAGATGGTAATATAAAAGCAAGAAGTATTTTTTTTTTG
TAATGACTGAAAGCTGTTCTGTGGATGACCTACCCTTTCCTTTAAACACGATTCTCTCACTTCCAACTCC
AAACTTGCTCAACTAATCCTTAAAAATAAACTTGAGCTGGAATTTG

SEQ ID NO:2

>gi|18597006|ref|XP_085119.1| similar to glucosamine-phosphate N-acetyltransferase 1; glucosamine-6-phosphate acetyltransferase; glucosamine-phosphate N-acetyltransferase [Homo sapiens]
MKPDETPMFDPSLLKEVDWSQNTATFSPAISPTHPGEGLVLRPLCTADLNRGFFKVLGQLTETGVVSPEQ
FMKSFEHMKKSGDYYVTVVEDVTLGQIVATATLIIEHKFIHSCAKRGRVEDVVVSDECRGKQLGKLLLST
LTLLSKKLNCYKITLECLPQNVGFYKKFGYTVSEENYMCRRFLK

7

An das Europäische Patentamt | To the European Patent Office | A l'Office européen des brevets 1

Eintritt in die europäische Phase (EPA als Bestimmungsamt oder ausgewähltes Amt)

Entry into the European phase (EPO as designated or elected Office)

Entrée dans la phase européenne (l'OEB agissant en qualité d'office désigné ou élu)

Europäische Anmeldenummer oder, falls nicht bekannt, PCT-Aktenzeichen oder PCT-Veröffentlichungsnummer	European application number, or, if not known, PCT application or publication number 03800355.4	Numéro de dépôt de la demande de brevet européen ou, à défaut, numéro de dépôt PCT ou de publication PCT
Zeichen des Anmelders oder Vertreters (max. 15 Positionen)	Applicant's or representative's reference (max. 15 spaces) P40798-/GUYF/MCM	Référence du demandeur ou du mandataire (15 caractères ou espaces au maximum)

☒ **1. Anmelder**
Die Angaben über den (die) Anmelder sind in der internationalen Veröffentlichung enthalten oder vom Internationalen Büro nach der internationalen Veröffentlichung vermerkt worden.

☐ Änderungen, die das Internationale Büro noch nicht vermerkt hat, sind auf einem Zusatzblatt angegeben.

Zustellanschrift
(siehe Merkblatt II, 1)

1. Applicant
Indications concerning the applicant(s) are contained in the international publication or recorded by the International Bureau after the international publication.

Changes which have not yet been recorded by the International Bureau are set out on an additional sheet.

Address for correspondence
(see Notes II, 1)

1. Demandeur
Les indications concernant le(s) demandeur(s) figurent dans la publication internationale ou ont été enregistrées par le Bureau international après la publication internationale.

Les changements qui n'ont pas encore été enregistrés par le Bureau international sont indiqués sur une feuille additionnelle.

Adresse pour la correspondance
(voir notice II, 1)

2. Vertreter

Name (Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird)

Geschäftsanschrift

Telefon

Telefax **Telex**

2. Representative

Name (Name only one representative who will be listed in the Register of European Patents and to whom notification will be made)
MALCOLM C MAIN

Address of place of business
Murgitroyd & Company
165 - 169 Scotland House
Glasgow, G5 8PL
United Kingdom

Telephone
+44 (0) 141 307 8400

Fax **Telex**
+ 44 (0) 141 307 8401

2. Mandataire

Nom (N'indiquer qu'un seul mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

Adresse professionnelle

Téléphone

Téléfax **Télex**

☒ Weitere(r) Vertreter auf Zusatzblatt — Additional representative(s) on additional sheet — Autre(s) mandataire(s) sur une feuille additionnelle

3. Vollmacht

☐ Einzelvollmacht ist beigefügt.

☐ Allgemeine Vollmacht ist registriert unter Nummer:

☐ Allgemeine Vollmacht ist eingereicht, aber noch nicht registriert.

☐ Die beim EPA als PCT-Anmeldeamt eingereichte Vollmacht schließt ausdrücklich die europäische Phase ein.

3. Authorisation

Individual authorisation is attached.

General authorisation has been registered under No:

A general authorisation has been filed, but not yet registered.

The authorisation filed with the EPO as PCT receiving Office expressly includes the European phase.

3. Pouvoir

Un pouvoir spécial est joint.

Un pouvoir général a été enregistré sous le n° :

Un pouvoir général a été déposé, mais n'est pas encore enregistré.

Le pouvoir général déposé à l'OEB agissant en qualité d'office récepteur au titre du PCT s'applique expressément à la phase européenne.

Zur Kasse

☒	**4. Prüfungsantrag** Hiermit wird die Prüfung der Anmeldung gemäß Art. 94 EPÜ beantragt. Die Prüfungsgebühr wird (wurde) entrichtet.	**4. Request for examination** Examination of the application under Art. 94 EPC is hereby requested. The examination fee is being (has been, will be) paid.	**4. Requête en examen** Il est demandé que soit examinée la demande de brevet conformément à l'art. 94 CBE. Il est (a été, sera) procédé au paiement de la taxe d'examen.
	Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt III, 5.2) :	Request for examination in an admissible non-EPO language (see Notes III, 5.2) :	Requête en examen dans une langue non officielle autorisée (voir notice III, 5.2) :
☒	**5. Abschriften** Zusätzliche Abschrift(en) der im ergänzenden europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt.	**5. Copies** Additional copy (copies) of the documents cited in the supplementary European search report is (are) requested.	**5. Copies** Prière de fournir une ou plusieurs copies supplémentaires des documents cités dans le rapport complémentaire de recherche européenne.
	Anzahl der **zusätzlichen** Sätze von Abschriften	Number of **additional** sets of copies	Nombre de jeux **supplémentaires** de copies
		ONE	
	6. Für das Verfahren vor dem EPA bestimmte Unterlagen	**6. Documents intended for proceedings before the EPO**	**6. Pièces destinées à la procédure devant l'OEB**
	6.1 Dem Verfahren vor dem EPA als **Bestimmungsamt** (PCT I) sind folgende Unterlagen zugrunde zu legen:	6.1 Proceedings before the EPO as **designated Office** (PCT I) are to be based on the following documents:	6.1 La procédure devant l'OEB agissant en qualité d'**office désigné** (PCT I) doit se fonder sur les pièces suivantes :
☒	die vom Internationalen Büro **veröffentlichten Anmeldungsunterlagen** (mit allen Ansprüchen, Beschreibung und Zeichnungen), gegebenenfalls mit den geänderten Ansprüchen nach Art. 19 PCT	**the application documents published** by the International Bureau (with all claims, description and drawings), where applicable with amended claims under Art. 19 PCT	les **pièces de la demande publiée** par le Bureau international (avec **toutes** les revendications, la description et les dessins), éventuellement avec les revendications modifiées conformément à l'article 19 du PCT
☐	soweit sie nicht ersetzt werden durch die beigefügten **Änderungen.**	unless replaced by the **amendments** enclosed.	dans la mesure où elles ne sont pas remplacées par les **modifications** jointes.
	Falls nötig, sind Klarstellungen auf einem Zusatzblatt einzureichen!	*Where necessary, clarifications must be submitted on a separate sheet!*	*Le cas échéant, des explications doivent être jointes sur une feuille additionnelle!*
	6.2 Dem Verfahren vor dem EPA als **ausgewähltem Amt** (PCT II) sind folgende Unterlagen zugrunde zu legen:	6.2 Proceedings before the EPO as **elected Office** (PCT II) are to be based on the following documents:	6.2 La procédure devant l'OEB **agissant en qualité d'office élu** (PCT II) doit se fonder sur les pièces suivantes :
☒	die dem **internationalen vorläufigen Prüfungsbericht zugrunde gelegten Unterlagen,** einschließlich seiner eventuellen **Anlagen** *(Solche Anlagen müssen immer beigefügt werden)*	**the documents on which the international preliminary examination report is based,** including its possible **annexes** *(Such annexes must always be filed)*	**les pièces sur lesquelles se fonde le rapport d'examen préliminaire international,** y compris ses **annexes** éventuelles *(De telles annexes sont toujours à joindre)*
☐	soweit sie nicht ersetzt werden durch die beigefügten **Änderungen.**	unless replaced by the **amendments** enclosed.	dans la mesure où elles ne sont pas remplacées par les **modifications** jointes.
	Falls nötig, sind Klarstellungen auf einem Zusatzblatt einzureichen!	*Where necessary, clarifications must be submitted on a seperate sheet!*	*Le cas échéant, des explications doivent être jointes sur une feuille additionnelle!*
☒	Sind dem EPA als mit der internationalen vorläufigen Prüfung beauftragten Behörde **Versuchsberichte** zugegangen, dürfen diese dem Verfahren vor dem EPA zugrunde gelegt werden.	If the EPO as International Preliminary Examining Authority has received **test reports**, these may be used as the basis of proceedings before the EPO.	Si l'OEB, agissant en qualité d'administration chargée de l'examen préliminaire international, a reçu des **rapports d'essais,** ceux-ci peuvent constituer la base de la procédure devant l'OEB.

7. Übersetzungen Beigefügt sind die nachfolgend angekreuzten Übersetzungen in einer der Amtssprachen des EPA (Deutsch, Englisch, Französisch):	**7. Translations** Translations in one of the official languages of the EPO (English, French, German) are enclosed as crossed below:	**7. Traductions** Vous trouverez, ci-joint, les traductions cochées ci-après dans l'une des langues officielles de l'OEB (allemand, anglais, français) :
• *Im Verfahren vor dem EPA als* ***Bestimmungsamt oder ausgewähltem Amt*** *(PCT I + II):*	• *In proceedings before the EPO as* ***designated or elected Office*** *(PCT I + II):*	• *Dans la procédure devant l'OEB agissant en qualité d'****office désigné ou élu*** *(PCT I + II):*
☐ Übersetzung der **ursprünglich eingereichten internationalen Anmeldung** (Beschreibung, Ansprüche, etwaige Textbestandteile in den Zeichnungen), der veröffentlichten Zusammenfassung, und etwaiger Angaben über biologisches Material nach Regel 13bis.3 und 13bis.4 PCT	Translation of the **international application** (description, claims, any text in the drawings) **as originally filed**, of the abstract as published and of any indication under Rule 13bis.3 and 13bis.4 PCT regarding biological material	Traduction de la **demande internationale telle que déposée initialement** (description, revendications, textes figurant éventuellement dans les dessins), de l'abrégé publié, et de toutes indications visées aux règles 13bis.3 et 13bis.4 du PCT concernant le matériel biologique
☐ Übersetzung der **prioritätsbegründenden Anmeldung(en)**	Translation of **the priority application(s)**	Traduction de la (des) **demande(s) ouvrant le droit de priorité**
☐ Es wird hiermit erklärt, daß die internationale Anmeldung in ihrer ursprünglich eingereichten Fassung eine vollständige Übersetzung der früheren Anmeldung ist (Regel 38(5) EPÜ)	It is hereby declared that the international application as originally filed is a complete translation of the previous application (Rule 38(5) EPC)	Il est déclaré par la présente que la demande internationale telle que déposée initialement est une traduction intégrale de la demande antérieure (règle 38(5) CBE)
• ***Zusätzlich*** *im Verfahren vor dem EPA als* ***Bestimmungsamt*** *(PCT I):*	• ***In addition,*** *in proceedings before the EPO as* ***designated Office*** *(PCT I):*	• ***De plus,*** *dans la procédure devant l'OEB agissant en qualité d'****office désigné*** *(PCT I) :*
☐ Übersetzung der nach Art. 19 PCT **geänderten Ansprüche** nebst Erklärung, falls diese dem Verfahren vor dem EPA zugrunde gelegt werden sollen (siehe Feld 6)	Translation of **amended claims** and any statement under Art. 19 PCT, if the claims as amended are to form the basis for the proceedings before the EPO (see Section 6)	Traduction des **revendications modifiées** et de la déclaration faite conformément à l'article 19 du PCT, si la procédure devant l'OEB doit être fondée sur les revendications modifiées (voir la rubrique 6)
• ***Zusätzlich*** *im Verfahren vor dem EPA als* ***ausgewähltem Amt*** *(PCT II):*	• ***In addition,*** *in proceedings before the EPO as* ***elected Office*** *(PCT II):*	• ***De plus,*** *dans la procédure devant l'OEB agissant en qualité d'****office élu*** *(PCT II) :*
☐ Übersetzung der **Anlagen zum internationalen vorläufigen Prüfungsbericht**	Translation of any **annexes to the international preliminary examination report**	Traduction des **annexes du rapport d'examen préliminaire international**

☐ **8. Biologisches Material** Die Erfindung bezieht sich auf bzw. verwendet biologisches Material, das nach Regel 28 EPÜ hinterlegt worden ist.	**8. Biological material** The invention relates to and/or uses biological material deposited under Rule 28 EPC.	**8. Matière biologique** L'invention concerne et/ou utilise de la matière biologique, déposée conformément à la règle 28 CBE.
☐ Die **Angaben nach Regel 28(1)c) EPÜ** (falls noch nicht bekannt, die Hinterlegungsstelle und das (die) Bezugszeichen (Nummer, Symbole usw.) des Hinterlegers) sind in der internationalen Veröffentlichung oder in der gemäß Feld 7 eingereichten Übersetzung enthalten auf:	**The particulars referred to in Rule 28(1)(c) EPC** (if not yet known, the depository institution and the identification reference(s) (number, symbols etc.) of the depositor) are given in the international publication or in the translation submitted under Section 7 on:	**Les indications visées à la règle 28(1)c) CBE** (si non encore connues, l'autorité de dépôt et la (les) référence(s) d'identification (numéro ou symboles etc.) du déposant) figurent dans la publication internationale ou dans une traduction produite conformément à la rubrique 7 à la / aux
Seite(n) / Zeile(n)	page(s) / line(s)	page(s) / ligne(s)
Die **Empfangsbescheinigung(en)** der Hinterlegungsstelle	The **receipt(s) of deposit** issued by the depositary institution	Le(s) **récépissé(s) de dépôt** délivré(s) par l'autorité de dépôt
☐ ist (sind) beigefügt	is (are) enclosed	est (sont) joint(s)
☐ wird (werden) nachgereicht	will be filed at a later date	sera (seront) produit(s) ultérieurement
☐ Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) EPÜ auf gesondertem Schriftstück	Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) EPC attached.	Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3) CBE.

9. Nucleotid- und Aminosäuresequenzen	**9. Nucleotide and amino acid sequences**	**9. Séquences de nucléotides et d'acides aminés**
☐ Die nach Regeln 5.2 und 13ter PCT sowie Regel 111(3) EPÜ erforderlichen Unterlagen liegen dem EPA bereits vor.	The items necessary in accordance with Rules 5.2 and 13ter PCT and Rule 111(3) EPC have already been furnished to the EPO.	Les pièces requises selon les règles 5.2 et 13ter PCT et la règle 111(3) CBE ont déjà été déposées auprès de l'OEB.
☐ Das schriftliche Sequenzprotokoll wird anliegend nachgereicht.	The written sequence listing is furnished herewith.	La liste de séquences écrite est produite ci-joint.
☐ Das Sequenzprotokoll geht nicht über den Inhalt der Anmeldung in der ursprünglich eingereichten Fassung hinaus.	The sequence listing does not include matter which goes beyond the content of the application as filed.	La liste de séquences ne contient pas d'éléments s'étendant au-delà du contenu de la demande telle qu'elle a été déposée.
☒ Der vorgeschriebene Datenträger ist beigefügt.	The prescribed data carrier is enclosed.	Le support de données prescrit est joint.
☒ Die auf dem Datenträger gespeicherte Information stimmt mit dem schriftlichen Sequenzprotokoll überein.	The information recorded on the data carrier is identical to the written sequence listing.	L'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite.

10. Benennungsgebühren	**10. Designation fees**	**10. Taxes de désignation**
☒ 10.1 Es ist derzeit beabsichtigt, den **siebenfachen** Betrag einer Benennungsgebühr zu entrichten. Damit gelten die Benennungsgebühren für **alle Vertragsstaaten des EPÜ**[1] als entrichtet (Art. 2 Nr. 3 GebO), soweit sie **in der internationalen Anmeldung** bestimmt sind[2].	10.1 It is currently intended to pay **seven times** the amount of the designation fee. The designation fees for **all the EPC contracting states**[1] **designated in the international application**[2] are thereby deemed to have been paid (Art. 2 No. 3 RFees).	10.1 Il est actuellement envisagé de payer un montant correspondant à **sept fois** la taxe de désignation. Les taxes de désignation sont ainsi réputées payées pour **tous les Etats contractants de la CBE**[1] **désignés dans la demande internationale**[2] (art. 2, point 3 du RRT).
☐ 10.2 Abweichend von der Erklärung in Nr. 10.1 ist derzeit beabsichtigt, **weniger als sieben** Benennungsgebühren für folgende **in der internationalen Anmeldung bestimmte Vertragsstaaten des EPÜ**[2] zu entrichten:	10.2 The declaration in No. 10.1 does not apply. Instead, it is currently intended to pay **fewer than seven** designation fees for the following **EPC contracting states**[2] **designated in the international application:**	10.2 Contrairement à ce qui est indiqué au n° 10.1, il est actuellement envisagé de payer **moins de sept** taxes de désignation pour les **Etats contractants de la CBE**[2] suivants **désignés dans la demande internationale** :
(1) ☐ _____ (2) ☐ _____ (3) ☐ _____		(4) ☐ _____ (5) ☐ _____ (6) ☐ _____
Soweit unter Nr. 10.2 Vertragsstaaten aufgeführt sind, wird beantragt, für die dort nicht aufgeführten Vertragsstaaten von der Zustellung einer Mitteilung nach Regel 108(3) EPÜ abzusehen.	If contracting states are indicated under No. 10.2, it is requested that no communication under Rule 108(3) EPC be issued for contracting states not thus indicated.	Si des Etats contractants sont mentionnés au n° 10.2, prière de ne pas procéder à la signification d'une notification prévue par la règle 108(3) CBE pour les Etats contractants n'y étant pas mentionnés.
☒ 10.3 Wird ein **automatischer Abbuchungsauftrag** erteilt (Feld 12), so wird das EPA beauftragt, bei Ablauf der Grundfrist nach Regel 107 (1)(d) EPÜ den siebenfachen Betrag einer Benennungsgebühr abzubuchen. Ist eine Erklärung nach Nr. 10.2 abgegeben worden, so sollen die Benennungsgebühren nur für die dort angegebenen Vertragsstaaten abgebucht werden, sofern dem EPA nicht bis zum Ablauf der Grundfrist ein anderslautender Auftrag zugeht.	10.3 If an **automatic debit order** has been issued (Section 12), the EPO is authorised, on expiry of the basic period under Rule 107(1)(d) EPC, to debit seven times the amount of the designation fee. If states are indicated under No. 10.2, the EPO will debit designation fees only for those states, unless instructed otherwise before the basic period expires.	10.3 Si un **ordre de prélèvement automatique** est donné (rubrique 12), il est demandé à l'OEB de prélever, à l'expiration du délai normal visé à la règle 107(1)d) CBE, un montant correspondant à sept fois la taxe de désignation. Si une déclaration a été faite au n° 10.2, les taxes de désignation ne sont à prélever que pour les Etats contractants qui y sont indiqués, sauf instruction contraire reçue par l'OEB avant l'expiration du délai normal.

1 Stand bei Drucklegung: 27 Vertragsstaaten, und zwar: / Status when this form was printed: 27 contracting states, namely / Situation à la date d'impression : 27 Etats contractants, à savoir : AT Österreich / Austria / Autriche, BE Belgien / Belgium / Belgique, BG Bulgarien / Bulgaria / Bulgarie, CH / LI Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein, CY Zypern / Cyprus / Chypre, CZ Tschechische Republik / Czech Republic / République tchèque, DE Deutschland / Germany / Allemagne, DK Dänemark / Denmark / Danemark, EE Estland / Estonia / Estonie, ES Spanien / Spain / Espagne, FI Finnland / Finland / Finlande, FR Frankreich / France / France, GB Vereinigtes Königreich / United Kingdom / Royaume-Uni, GR Griechenland / Greece / Grèce, HU Ungarn / Hungary / Hongrie, IE Irland / Ireland / Irlande, IT Italien / Italy / Italie, LU Luxemburg / Luxembourg / Luxembourg, MC Monaco / Monaco / Monaco, NL Niederlande / Netherlands / Pays-Bas, PT Portugal / Portugal / Portugal, RO Rumänien / Romania / Roumanie, SE Schweden / Sweden / Suède, SI Slowenien / Slovenia / Slovénie, SK Slowakische Republik / Slovak Republic / République slovaque, TR Türkei / Turkey / Turquie

2 Für folgende Staaten nur möglich, falls in der internationalen Anmeldung am oder nach folgendem Tag bestimmt: Slowakische Republik, Bulgarien, Tschechische Republik und Estland: 1. Juli 2002, Slowenien: 1. Dezember 2002, Ungarn: 1. Januar 2003 und Rumänien: 1. März 2003. / For the following states this is possible only if they are designated in the international application on or after the stated date: Slovak Republic, Bulgaria, Czech Republic and Estonia: 1 July 2002, Slovenia: 1 December 2002, Hungary: 1 January 2003 and Romania: 1 March 2003. / En ce qui concerne les Etats suivants seulement si la désignation a été effectuée dans la demande internationale à la date suivante ou à une date ultérieure: République slovaque, Bulgarie, République tchèque et Estonie: 1er juillet 2002, Slovénie: 1er décembre 2002, Hongrie: 1er janvier 2003 et Roumanie: 1er mars 2003.

☒ **11. Erstreckung des europäischen Patents**
Bei Zahlung der Erstreckungsgebühr(en) gilt diese Anmeldung auch als wirksamer Erstreckungsantrag für die in der internationalen Anmeldung bestimmten »Erstreckungsstaaten«. Es ist beabsichtigt, diese Gebühr(en) für folgende Staaten zu entrichten:

11. Extension of the European patent
On payment of the extension fee(s) this application is also deemed to be a request for extension to all the "extension states" designated in the international application. It is intended to pay the fee(s) for the following states:

11. Extension des effets du brevet européen
La taxe (Les taxes) d'extension payée(s), la présente demande est également réputée être une demande d'extension à tous les «Etats autorisant l'extension» désignés dans la demande internationale. Il est envisagé de payer la taxe (les taxes) d'extension pour les Etats suivants:

☐	SI	Slowenien [1]	Slovenia [1]	Slovénie [1]
☒	LT	Litauen	Lithuania	Lituanie
☒	LV	Lettland	Latvia	Lettonie
☒	AL	Albanien	Albania	Albanie
☐	RO	Rumänien [1]	Romania [1]	Roumanie [1]
☒	MK	Ehemalige jugoslawische Republik Mazedonien	Former Yugoslav Republic of Macedonia	Ex-République yougoslave de Macédoine
☐	____	_____ [2]	_____ [2]	_____ [2]

1) Für Slowenien und Rumänien nur möglich, falls in der internationalen Anmeldung bis 30. November 2002 (Slowenien) oder bis 28. Februar 2003 (Rumänien) bestimmt. / For Slovenia and Romania this is possible only if they are designated in the international application up to 30 November 2002 (Slovenia) or 28 February 2003 (Romania). / En ce qui concerne la Slovénie et la Roumanie, seulement si la désignation a été effectuée dans la demande internationale jusqu'au 30 novembre 2002 (Slovénie) ou jusqu'au 28 février 2003 (Roumanie).

2) Platz für Staaten, mit denen »Erstreckungsabkommen« nach Drucklegung dieses Formblatts in Kraft treten und die in der internationalen Anmeldung bestimmt waren. / Space for States with which "extension agreements" enter into force after this form has been printed and which were designated in the international application. / Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire et qui ont été désignés dans la demande internationale.

12. Automatischer Abbuchungsauftrag ***(Nur möglich für Inhaber von beim EPA geführten laufenden Konten)***

☐ Das EPA wird beauftragt, nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren fällige Gebühren und Auslagen vom untenstehenden laufenden Konto abzubuchen. In Bezug auf die **Benennungsgebühren** wird auf Feld 10.3 verwiesen. Das EPA wird ferner beauftragt, die **Erstreckungsgebühren** für jeden in Feld 11 angekreuzten »Erstreckungsstaat« bei Ablauf der Grundfrist zu ihrer Zahlung abzubuchen, sofern ihm nicht bis dahin ein anderslautender Auftrag zugeht.

Nummer und Kontoinhaber

12. Automatic debit order ***(for EPO deposit account holders only)***

The EPO is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account below any fees and costs falling due. For **designation fees**, see Section 10.3. The EPO is also authorised, on expiry of the basic period for paying the **extension fees**, to debit those fees for each of the "extension states" marked with a cross in Section 11, unless instructed otherwise before the said period expires.

Number and account holder

12. Ordre de prélèvement automatique ***(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)***
Par la présente, il est demandé à l'OEB de prélever du compte courant ci-dessous les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique. Pour les **taxes de désignation**, se reporter à la rubrique 10.3. Il est en outre demandé à l'OEB de prélever, à l'expiration du délai normal prévu pour leur paiement, les **taxes d'extension** pour chaque «Etat autorisant l'extension» coché à la rubrique 11, sauf instruction contraire reçue avant l'expiration de ce délai.

Numéro et titulaire du compte

☒ **13.** Eventuelle **Rückzahlungen** auf das beim EPA geführte laufende Konto

Nummer und Kontoinhaber

13. Any **reimbursement** to EPO deposit account

Number and account holder

2805 0177 Murgitroyd & Company

13. Remboursements éventuels à effectuer sur le compte courant ouvert auprès de l'OEB
Numéro et titulaire du compte

14. Unterschrift(en) des (der) Anmelder(s) oder Vertreters

14. Signature(s) of applicant(s) or representative

Malcolm C Main

14. Signature(s) du (des) demandeur(s) ou du mandataire

Ort / Datum

Place / Date Glasgow, 11 May 2005

Lieu / Date

Für Angestellte (Art. 133(3) EPÜ) mit allgemeiner Vollmacht:

Nr.

Name(n) des (der) Unterzeichneten bitte in Druckschrift wiederholen. Bei juristischen Personen bitte auch die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft in Druckschrift angeben.

For employees (Art. 133(3) EPC) having a general authorisation:

No.

Please print name(s) under signature(s). In the case of legal persons, the position of the signatory within the company should also be printed.

Pour les employés (art. 133(3) CBE) disposant d'un pouvoir général :

N°

Le ou les noms des signataires doivent être indiqués en caractères d'imprimerie. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires doit également être indiquée en caractères d'imprimerie.

MURGITROYD & COMPANY

Representatives/ Mandataires/ Vertreter:

MURGITROYD, Ian	34200
PATTULLO, Norman	34710
COOPER, John	76420
OUZMAN, Beverley	76380
McNALLY, Roisin	86220
MURNANE, Graham	87840
EARNSHAW, Mark	75270
ALLAN, Jamie	90950
GORDON, Naoise	74290
McKAY, Jacqueline	95990
JONES, Keith	72980
BROWN, James	126270
MAIN, Malcolm	122920
DeZEEUW, Rick	125950
TEN BRINK, Carsten	95280
WALLER, Stephen	0136010
WATSON, Craig	143030
DRYSDALE, Douglas	143820
CORET, Sophie	144560
D'ARCY, Julia	143810
ANDERSON, Angela	078500

File Ref: P40798-/GUYF/MCM

11 May 2005

MURGITROYD
& COMPANY
European Patent and Trade Mark Attorneys
ABERDEEN · BELFAST · DUBLIN
GLASGOW · LONDON
MUNICH · MÜNSTER · NICE

Your Ref: - - -
Our Ref: P40798-/GUYF/MCM

European Patent Office
D-80298 Munich
Germany

ADVANCED COPY VIA FACSIMILE NO 00 49 89 2399 4465 (9 PAGES)
CONFIRMATION COPY VIA COURIER

Dear Sirs

European Patent Application No 03800355.4
EPO Phase of International Application PCT/US2003/041619
Exelixis, Inc.
IP Title: FLJ10607 as Modifier of the Axin Pathway and Methods of Use

We enclose the following documents:-

1. EPO Form 1200 (5 pages + supplementary sheet).
2. Sequence listing in computer readable form (via courier only). The information recorded on the data carrier is identical to the written sequence.
3. Fee Sheet instructing debit of EUR 3695 from deposit account number 2805 0177 in respect of National Basic fee, Search Fee (Less 20%), seven Designation Fees in respect of all Contracting States, and Extension Fees in respect of Lithuania, Albania, Latvia and The former Yugoslav Republic of Macedonia, Claims Fees for claims 11 to 25, Examination fee and Additional Copies Fee.
4. EPO Form 1037 for receipt and return.

The originally filed Request Form, Description, Claims, Abstract, Drawings and Priority Document will already be in your possession via the International Bureau.

Yours faithfully
for Murgitroyd & Company

MALCOLM MAIN
malcolm.main@murgitroyd.com

Enc: As Above

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An das Europäische Patentamt | **To the European Patent Office** | **A l'Office européen des brevets** 1

EPO - Munich
29

Eintritt in die europäische Phase (EPA als Bestimmungsamt oder ausgewähltes Amt)

Entry into the European phase (EPO as designated or elected Office)

12 Mai 2005

Entrée dans la phase européenne (l'OEB agissant en qualité d'office désigné ou élu)

Europäische Anmeldenummer oder, falls nicht bekannt, PCT-Aktenzeichen oder PCT-Veröffentlichungsnummer	European application number, or, if not known, PCT application or publication number 03800355.4	Numéro de dépôt de la demande de brevet européen ou, à défaut, numéro de dépôt PCT ou de publication PCT
Zeichen des Anmelders oder Vertreters (max. 15 Positionen)	Applicant's or representative's reference (max. 15 spaces) P40798-/GUYF/MCM	Référence du demandeur ou du mandataire (15 caractères ou espaces au maximum) Zur Kasse A. € 3.695

☒ **1. Anmelder**
Die Angaben über den (die) Anmelder sind in der internationalen Veröffentlichung enthalten oder vom Internationalen Büro nach der internationalen Veröffentlichung vermerkt worden.

☐ Änderungen, die das Internationale Büro noch nicht vermerkt hat, sind auf einem Zusatzblatt angegeben.

Zustellanschrift
(siehe Merkblatt II, 1)

1. Applicant
Indications concerning the applicant(s) are contained in the international publication or recorded by the International Bureau after the international publication.

Changes which have not yet been recorded by the International Bureau are set out on an additional sheet.

Address for correspondence
(see Notes II, 1)

1. Demandeur
Les indications concernant le(s) demandeur(s) figurent dans la publication internationale ou ont été enregistrées par le Bureau international après la publication internationale.

Les changements qui n'ont pas encore été enregistrés par le Bureau international sont indiqués sur une feuille additionnelle.

Adresse pour la correspondance
(voir notice II, 1)

2. Vertreter

Name (Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird)

Geschäftsanschrift

Telefon

Telefax **Telex**

2. Representative

Name (Name only one representative who will be listed in the Register of European Patents and to whom notification will be made)

MALCOLM C MAIN

Address of place of business
Murgitroyd & Company
165 - 169 Scotland House
Glasgow, G5 8PL
United Kingdom

Telephone
+44 (0) 141 307 8400

Fax **Telex**
+ 44 (0) 141 307 8401

2. Mandataire

Nom (N'indiquer **qu' un seul** mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

Adresse professionnelle

Téléphone

Téléfax **Télex**

☒ Weitere(r) Vertreter auf Zusatzblatt

Additional representative(s) on additional sheet

Autre(s) mandataire(s) sur une feuille additionnelle

3. Vollmacht

☐ Einzelvollmacht ist beigefügt.

☐ Allgemeine Vollmacht ist registriert unter Nummer:

☐ Allgemeine Vollmacht ist eingereicht, aber noch nicht registriert.

☐ Die beim EPA als PCT-Anmeldeamt eingereichte Vollmacht schließt ausdrücklich die europäische Phase ein.

3. Authorisation

Individual authorisation is attached.

General authorisation has been registered under No:

A general authorisation has been filed, but not yet registered.

The authorisation filed with the EPO as PCT receiving Office expressly includes the European phase.

3. Pouvoir

Un pouvoir spécial est joint.

Un pouvoir général a été enregistré sous le n° :

Un pouvoir général a été déposé, mais n'est pas encore enregistré.

Le pouvoir général déposé à l'OEB agissant en qualité d'office récepteur au titre du PCT s'applique expressément à la phase européenne.

☒ **4. Prüfungsantrag**
Hiermit wird die Prüfung der Anmeldung gemäß Art. 94 EPÜ beantragt. Die Prüfungsgebühr wird (wurde) entrichtet.

Prüfungsantrag in einer zugelassenen Nichtamtssprache (siehe Merkblatt III, 5.2) :

4. Request for examination
Examination of the application under Art. 94 EPC is hereby requested. The examination fee is being (has been, will be) paid.

Request for examination in an admissible non-EPO language (see Notes III, 5.2) :

4. Requête en examen
Il est demandé que soit examinée la demande de brevet conformément à l'art. 94 CBE. Il est (a été, sera) procédé au paiement de la taxe d'examen.

Requête en examen dans une langue non officielle autorisée (voir notice III, 5.2) :

☒ **5. Abschriften**
Zusätzliche Abschrift(en) der im ergänzenden europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt.

Anzahl der **zusätzlichen** Sätze von Abschriften

5. Copies
Additional copy (copies) of the documents cited in the supplementary European search report is (are) requested.

Number of **additional** sets of copies

5. Copies
Prière de fournir une ou plusieurs copies supplémentaires des documents cités dans le rapport complémentaire de recherche européenne.

Nombre de jeux **supplémentaires** de copies

ONE

6. Für das Verfahren vor dem EPA bestimmte Unterlagen

6.1 Dem Verfahren vor dem EPA als **Bestimmungsamt** (PCT I) sind folgende Unterlagen zugrunde zu legen:

☒ die vom Internationalen Büro **veröffentlichten Anmeldungsunterlagen** (mit **allen** Ansprüchen, Beschreibung und Zeichnungen), gegebenenfalls mit den geänderten Ansprüchen nach Art. 19 PCT

☐ soweit sie nicht ersetzt werden durch die beigefügten **Änderungen.**

Falls nötig, sind Klarstellungen auf einem Zusatzblatt einzureichen!

6.2 Dem Verfahren vor dem EPA als **ausgewähltem Amt** (PCT II) sind folgende Unterlagen zugrunde zu legen:

☒ die dem **internationalen vorläufigen Prüfungsbericht zugrunde gelegten Unterlagen,** einschließlich seiner eventuellen **Anlagen**
(Solche Anlagen müssen immer beigefügt werden)

☐ soweit sie nicht ersetzt werden durch die beigefügten **Änderungen.**

Falls nötig, sind Klarstellungen auf einem Zusatzblatt einzureichen!

☒ Sind dem EPA als mit der internationalen vorläufigen Prüfung beauftragten Behörde **Versuchsberichte** zugegangen, dürfen diese dem Verfahren vor dem EPA zugrunde gelegt werden.

6. Documents intended for proceedings before the EPO

6.1 Proceedings before the EPO as **designated Office** (PCT I) are to be based on the following documents:

the **application documents published** by the International Bureau (with **all** claims, description and drawings), where applicable with amended claims under Art. 19 PCT

unless replaced by the **amendments** enclosed.

Where necessary, clarifications must be submitted on a separate sheet!

6.2 Proceedings before the EPO as **elected Office** (PCT II) are to be based on the following documents:

the **documents on which the international preliminary examination report is based,** including its possible **annexes**
(Such annexes must always be filed)

unless replaced by the **amendments** enclosed.

Where necessary, clarifications must be submitted on a separate sheet!

If the EPO as International Preliminary Examining Authority has received **test reports,** these may be used as the basis of proceedings before the EPO.

6. Pièces destinées à la procédure devant l'OEB

6.1 La procédure devant l'OEB agissant en qualité **d'office désigné** (PCT I) doit se fonder sur les pièces suivantes :

les **pièces de la demande publiée** par le Bureau international (avec **toutes** les revendications, la description et les dessins), éventuellement avec les revendications modifiées conformément à l'article 19 du PCT

dans la mesure où elles ne sont pas remplacées par les **modifications** jointes.

Le cas échéant, des explications doivent être jointes sur une feuille additionnelle!

6.2 La procédure devant l'OEB **agissant en qualité d'office élu** (PCT II) doit se fonder sur les pièces suivantes :

les **pièces sur lesquelles se fonde le rapport d'examen préliminaire international,** y compris ses **annexes** éventuelles
(De telles annexes sont toujours à joindre)

dans la mesure où elles ne sont pas remplacées par les **modifications** jointes.

Le cas échéant, des explications doivent être jointes sur une feuille additionnelle!

Si l'OEB, agissant en qualité d'administration chargée de l'examen préliminaire international, a reçu des **rapports d'essais,** ceux-ci peuvent constituer la base de la procédure devant l'OEB.

	7. Übersetzungen Beigefügt sind die nachfolgend angekreuzten Übersetzungen in einer der Amtssprachen des EPA (Deutsch, Englisch, Französisch):	**7. Translations** Translations in one of the official languages of the EPO (English, French, German) are enclosed as crossed below:	**7. Traductions** Vous trouverez, ci-joint, les traductions cochées ci-après dans l'une des langues officielles de l'OEB (allemand, anglais, français) :
	• *Im Verfahren vor dem EPA als* ***Bestimmungsamt oder ausgewähltem Amt*** *(PCT I + II):*	• *In proceedings before the EPO as* ***designated or elected Office*** *(PCT I + II):*	• *Dans la procédure devant l'OEB agissant en qualité d'****office désigné ou élu*** *(PCT I + II):*
☐	Übersetzung der **ursprünglich eingereichten internationalen Anmeldung** (Beschreibung, Ansprüche, etwaige Textbestandteile in den Zeichnungen), der veröffentlichten Zusammenfassung, und etwaiger Angaben über biologisches Material nach Regel 13bis.3 und 13bis.4 PCT	Translation of the **international application** (description, claims, any text in the drawings) **as originally filed**, of the abstract as published and of any indication under Rule 13bis.3 and 13bis.4 PCT regarding biological material	Traduction de la **demande internationale telle que déposée initialement** (description, revendications, textes figurant éventuellement dans les dessins), de l'abrégé publié, et de toutes indications visées aux règles 13bis.3 et 13bis.4 du PCT concernant le matériel biologique
☐	Übersetzung der **prioritätsbegründenden Anmeldung(en)**	Translation of **the priority application(s)**	Traduction de la (des) **demande(s) ouvrant le droit de priorité**
☐	Es wird hiermit erklärt, daß die internationale Anmeldung in ihrer ursprünglich eingereichten Fassung eine vollständige Übersetzung der früheren Anmeldung ist (Regel 38(5) EPÜ)	It is hereby declared that the international application as originally filed is a complete translation of the previous application (Rule 38(5) EPC)	Il est déclaré par la présente que la demande internationale telle que déposée initialement est une traduction intégrale de la demande antérieure (règle 38(5) CBE)
	• ***Zusätzlich*** *im Verfahren vor dem EPA als* ***Bestimmungsamt*** *(PCT I):*	• ***In addition,*** *in proceedings before the EPO as* ***designated Office*** *(PCT I):*	• ***De plus,*** *dans la procédure devant l'OEB agissant en qualité d'****office désigné*** *(PCT I) :*
☐	Übersetzung der nach Art. 19 PCT **geänderten Ansprüche** nebst Erklärung, falls diese dem Verfahren vor dem EPA zugrunde gelegt werden sollen (siehe Feld 6)	Translation of **amended claims** and any statement under Art. 19 PCT, if the claims as amended are to form the basis for the proceedings before the EPO (see Section 6)	Traduction des **revendications modifiées** et de la déclaration faite conformément à l'article 19 du PCT, si la procédure devant l'OEB doit être fondée sur les revendications modifiées (voir la rubrique 6)
	• ***Zusätzlich*** *im Verfahren vor dem EPA als* ***ausgewähltem Amt*** *(PCT II):*	• ***In addition,*** *in proceedings before the EPO as* ***elected Office*** *(PCT II):*	• ***De plus,*** *dans la procédure devant l'OEB agissant en qualité d'****office élu*** *(PCT II) :*
☐	Übersetzung der **Anlagen zum internationalen vorläufigen Prüfungsbericht**	Translation of any **annexes to the international preliminary examination report**	Traduction des **annexes du rapport d'examen préliminaire international**
☐	**8. Biologisches Material** Die Erfindung bezieht sich auf bzw. verwendet biologisches Material, das nach Regel 28 EPÜ hinterlegt worden ist.	**8. Biological material** The invention relates to and/or uses biological material deposited under Rule 28 EPC.	**8. Matière biologique** L'invention concerne et/ou utilise de la matière biologique, déposée conformément à la règle 28 CBE.
☐	Die **Angaben nach Regel 28(1)c) EPÜ** (falls noch nicht bekannt, die Hinterlegungsstelle und das (die) Bezugszeichen [Nummer, Symbole usw.] des Hinterlegers) sind in der internationalen Veröffentlichung oder in der gemäß Feld 7 eingereichten Übersetzung enthalten auf: Seite(n) / Zeile(n)	The **particulars referred to in Rule 28(1)(c) EPC** (if not yet known, the depository institution and the identification reference(s) [number, symbols etc.] of the depositor) are given in the international publication or in the translation submitted under Section 7 on: page(s) / line(s)	Les **indications visées à la règle 28(1)c) CBE** (si non encore connues, l'autorité de dépôt et la (les) référence(s) d'identification [numéro ou symboles etc.] du déposant) figurent dans la publication internationale ou dans une traduction produite conformément à la rubrique 7 à la / aux: page(s) / ligne(s)
	Die **Empfangsbescheinigung(en)** der Hinterlegungsstelle	The **receipt(s) of deposit** issued by the depositary institution	Le(s) **récépissé(s) de dépôt** délivré(s) par l'autorité de dépôt
☐	ist (sind) beigefügt	is (are) enclosed	est (sont) joint(s)
☐	wird (werden) nachgereicht	will be filed at a later date	sera (seront) produit(s) ultérieurement
☐	Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) EPÜ auf gesondertem Schriftstück	Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) EPC attached.	Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3) CBE.

9. Nucleotid- und Aminosäuresequenzen

☐ Die nach Regeln 5.2 und 13ter PCT sowie Regel 111(3) EPÜ erforderlichen Unterlagen liegen dem EPA bereits vor.

☐ Das schriftliche Sequenzprotokoll wird anliegend nachgereicht.

☐ Das Sequenzprotokoll geht nicht über den Inhalt der Anmeldung in der ursprünglich eingereichten Fassung hinaus.

☒ Der vorgeschriebene Datenträger ist beigefügt.

☒ Die auf dem Datenträger gespeicherte Information stimmt mit dem schriftlichen Sequenzprotokoll überein.

9. Nucleotide and amino acid sequences

The items necessary in accordance with Rules 5.2 and 13ter PCT and Rule 111(3) EPC have already been furnished to the EPO.

The written sequence listing is furnished herewith.

The sequence listing does not include matter which goes beyond the content of the application as filed.

The prescribed data carrier is enclosed.

The information recorded on the data carrier is identical to the written sequence listing.

9. Séquences de nucléotides et d'acides aminés

Les pièces requises selon les règles 5.2 et 13ter PCT et la règle 111(3) CBE ont déjà été déposées auprès de l'OEB.

La liste de séquences écrite est produite ci-joint.

La liste de séquences ne contient pas d'éléments s'étendant au-delà du contenu de la demande telle qu'elle a été déposée.

Le support de données prescrit est joint.

L'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite.

10. Benennungsgebühren

☒ 10.1 Es ist derzeit beabsichtigt, den **siebenfachen** Betrag einer Benennungsgebühr zu entrichten. Damit gelten die Benennungsgebühren für **alle Vertragsstaaten des EPÜ**[1] als entrichtet (Art. 2 Nr. 3 GebO), soweit sie **in der internationalen Anmeldung** bestimmt sind[2].

☐ 10.2 Abweichend von der Erklärung in Nr. 10.1 ist derzeit beabsichtigt, **weniger als sieben** Benennungsgebühren für folgende **in der internationalen Anmeldung bestimmte Vertragsstaaten des EPÜ**[2] zu entrichten:

(1) ☐ _____
(2) ☐ _____
(3) ☐ _____

Soweit unter Nr. 10.2 Vertragsstaaten aufgeführt sind, wird beantragt, für die dort nicht aufgeführten Vertragsstaaten von der Zustellung einer Mitteilung nach Regel 108(3) EPÜ abzusehen.

☒ 10.3 Wird ein **automatischer Abbuchungsauftrag** erteilt (Feld 12), so wird das EPA beauftragt, bei Ablauf der Grundfrist nach Regel 107 (1)d) EPÜ den siebenfachen Betrag einer Benennungsgebühr abzubuchen. Ist eine Erklärung nach Nr. 10.2 abgegeben worden, so sollen die Benennungsgebühren nur für die dort angegebenen Vertragsstaaten abgebucht werden, sofern dem EPA nicht bis zum Ablauf der Grundfrist ein anderslautender Auftrag zugeht.

10. Designation fees

10.1 It is currently intended to pay **seven times** the amount of the designation fee. The designation fees for **all the EPC contracting states[1] designated in the international application[2]** are thereby deemed to have been paid (Art. 2 No. 3 RFees).

10.2 The declaration in No. 10.1 does not apply. Instead, it is currently intended to pay **fewer than seven** designation fees for the following **EPC contracting states[2] designated in the international application**:

(4) ☐ _____
(5) ☐ _____
(6) ☐ _____

If contracting states are indicated under No. 10.2, it is requested that no communication under Rule 108(3) EPC be issued for contracting states not thus indicated.

10.3 If an **automatic debit order** has been issued (Section 12), the EPO is authorised, on expiry of the basic period under Rule 107(1)(d) EPC, to debit seven times the amount of the designation fee. If states are indicated under No. 10.2, the EPO will debit designation fees only for those states, unless instructed otherwise before the basic period expires.

10. Taxes de désignation

10.1 Il est actuellement envisagé de payer un montant correspondant à **sept fois** la taxe de désignation. Les taxes de désignation sont ainsi réputées payées pour **tous les Etats contractants de la CBE[1] désignés dans la demande internationale[2]** (art. 2, point 3 du RRT).

10.2 Contrairement à ce qui est indiqué au n° 10.1, il est actuellement envisagé de payer **moins de sept** taxes de désignation pour les **Etats contractants de la CBE[2]** suivants **désignés dans la demande internationale** :

Si des Etats contractants sont mentionnés au n° 10.2, prière de ne pas procéder à la signification d'une notification prévue par la règle 108(3) CBE pour les Etats contractants n'y étant pas mentionnés.

10.3 Si un **ordre de prélèvement automatique** est donné (rubrique 12), il est demandé à l'OEB de prélever, à l'expiration du délai normal visé à la règle 107(1)d) CBE, un montant correspondant à sept fois la taxe de désignation. Si une déclaration a été faite au n° 10.2, les taxes de désignation ne sont à prélever que pour les Etats contractants qui y sont indiqués, sauf instruction contraire reçue par l'OEB avant l'expiration du délai normal.

1 Stand bei Drucklegung: 27 Vertragsstaaten, und zwar: / Status when this form was printed: 27 contracting states, namely / Situation à la date d'impression : 27 Etats contractants, à savoir : AT Österreich / Austria / Autriche, BE Belgien / Belgium / Belgique, BG Bulgarien / Bulgaria / Bulgarie, CH / LI Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein, CY Zypern / Cyprus / Chypre, CZ Tschechische Republik / Czech Republic / République tchèque, DE Deutschland / Germany / Allemagne, DK Dänemark / Denmark / Danemark, EE Estland / Estonia / Estonie, ES Spanien / Spain / Espagne, FI Finnland / Finland / Finlande, FR Frankreich / France / France, GB Vereinigtes Königreich / United Kingdom / Royaume-Uni, GR Griechenland / Greece / Grèce, HU Ungarn / Hungary / Hongrie, IE Irland / Ireland / Irlande, IT Italien / Italy / Italie, LU Luxemburg / Luxembourg / Luxembourg, MC Monaco / Monaco / Monaco, NL Niederlande / Netherlands / Pays-Bas, PT Portugal / Portugal / Portugal, RO Rumänien / Romania / Roumanie, SE Schweden / Sweden / Suède, SI Slowenien / Slovenia / Slovénie, SK Slowakische Republik / Slovak Republic / République slovaque, TR Türkei / Turkey / Turquie

2 Für folgende Staaten nur möglich, falls in der internationalen Anmeldung am oder nach folgendem Tag bestimmt: Slowakische Republik, Bulgarien, Tschechische Republik und Estland: 1. Juli 2002, Slowenien: 1. Dezember 2002, Ungarn: 1. Januar 2003 und Rumänien: 1. März 2003. / For the following states this is possible only if they are designated in the international application on or after the stated date: Slovak Republic, Bulgaria, Czech Republic and Estonia: 1 July 2002, Slovenia: 1 December 2002, Hungary: 1 January 2003 and Romania: 1 March 2003. / En ce qui concerne les Etats suivants seulement si la désignation a été effectuée dans la demande internationale à la date suivante ou à une date ultérieure: République slovaque, Bulgarie, République tchèque et Estonie: 1er juillet 2002, Slovénie: 1er décembre 2002, Hongrie: 1er janvier 2003 et Roumanie: 1er mars 2003.

☒ **11. Erstreckung des europäischen Patents**
Bei Zahlung der Erstreckungsgebühr(en) gilt diese Anmeldung auch als wirksamer Erstreckungsantrag für die in der internationalen Anmeldung bestimmten »Erstreckungsstaaten«. Es ist beabsichtigt, diese Gebühr(en) für folgende Staaten zu entrichten:

11. Extension of the European patent
On payment of the extension fee(s) this application is also deemed to be a request for extension to all the "extension states" designated in the international application. It is intended to pay the fee(s) for the following states:

11. Extension des effets du brevet européen
La taxe (Les taxes) d'extension payée(s), la présente demande est également réputée être une demande d'extension à tous les «Etats autorisant l'extension» désignés dans la demande internationale. Il est envisagé de payer la taxe (les taxes) d'extension pour les Etats suivants:

☐	SI	Slowenien [1]	Slovenia [1]	Slovénie [1]
☒	LT	Litauen	Lithuania	Lituanie
☒	LV	Lettland	Latvia	Lettonie
☒	AL	Albanien	Albania	Albanie
☐	RO	Rumänien [1]	Romania [1]	Roumanie [1]
☒	MK	Ehemalige jugoslawische Republik Mazedonien	Former Yugoslav Republic of Macedonia	Ex-République yougoslave de Macédoine
☐	____	_____ [2]	_____ [2]	_____ [2]

1) Für Slowenien und Rumänien nur möglich, falls in der internationalen Anmeldung bis 30. November 2002 (Slowenien) oder bis 28. Februar 2003 (Rumänien) bestimmt. / For Slovenia and Romania this is possible only if they are designated in the international application up to 30 November 2002 (Slovenia) or 28 February 2003 (Romania). / En ce qui concerne la Slovénie et la Roumanie, seulement si la désignation a été effectuée dans la demande internationale jusqu'au 30 novembre 2002 (Slovénie) ou jusqu'au 28 février 2003 (Roumanie).

2) Platz für Staaten, mit denen »Erstreckungsabkommen« nach Drucklegung dieses Formblatts in Kraft treten und die in der internationalen Anmeldung bestimmt waren. / Space for States with which "extension agreements" enter into force after this form has been printed and which were designated in the international application. / Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire et qui ont été désignés dans la demande internationale.

12. Automatischer Abbuchungsauftrag ***(Nur möglich für Inhaber von beim EPA geführten laufenden Konten)***

☐ Das EPA wird beauftragt, nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren fällige Gebühren und Auslagen vom untenstehenden laufenden Konto abzubuchen. In Bezug auf die **Benennungsgebühren** wird auf Feld 10.3 verwiesen. Das EPA wird ferner beauftragt, die **Erstreckungsgebühren** für jeden in Feld 11 angekreuzten »Erstreckungsstaat« bei Ablauf der Grundfrist zu ihrer Zahlung abzubuchen, sofern ihm nicht bis dahin ein anderslautender Auftrag zugeht.

Nummer und Kontoinhaber

12. Automatic debit order ***(for EPO deposit account holders only)***

The EPO is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account below any fees and costs falling due. For **designation fees**, see Section 10.3. The EPO is also authorised, on expiry of the basic period for paying the **extension fees**, to debit those fees for each of the "extension states" marked with a cross in Section 11, unless instructed otherwise before the said period expires.

Number and account holder

12. Ordre de prélèvement automatique ***(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)***

Par la présente, il est demandé à l'OEB de prélever du compte courant ci-dessous les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique. Pour les **taxes de désignation**, se reporter à la rubrique 10.3. Il est en outre demandé à l'OEB de prélever, à l'expiration du délai normal prévu pour leur paiement, les **taxes d'extension** pour chaque «Etat autorisant l'extension» coché à la rubrique 11, sauf instruction contraire reçue avant l'expiration de ce délai.

Numéro et titulaire du compte

☒ **13.** Eventuelle **Rückzahlungen** auf das beim EPA geführte laufende Konto

Nummer und Kontoinhaber

13. Any **reimbursement** to EPO deposit account

Number and account holder

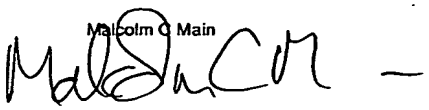
2805 0177 Murgitroyd & Company

13. Remboursements éventuels à effectuer sur le compte courant ouvert auprès de l'OEB
Numéro et titulaire du compte

14. Unterschrift(en) des (der) Anmelder(s) oder Vertreters

14. Signature(s) of applicant(s) or representative

Malcolm C Main



14. Signature(s) du (des) demandeur(s) ou du mandataire

Ort / Datum

Für Angestellte (Art. 133(3) EPÜ) mit allgemeiner Vollmacht:

Nr.

Name(n) des (der) Unterzeichneten bitte in Druckschrift wiederholen. Bei juristischen Personen bitte auch die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft in Druckschrift angeben.

Place / Date Glasgow, 11 May 2005

For employees (Art. 133(3) EPC) having a general authorisation:

No.

Please print name(s) under signature(s). In the case of legal persons, the position of the signatory within the company should also be printed.

Lieu / Date

Pour les employés (art. 133(3) CBE) disposant d'un pouvoir général :

N°

Le ou les noms des signataires doivent être indiqués en caractères d'imprimerie. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires doit également être indiquée en caractères d'imprimerie.

MURGITROYD
& COMPANY

Representatives/ Mandataires/ Vertreter:

MURGITROYD, Ian	34200
PATTULLO, Norman	34710
COOPER, John	76420
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McNALLY, Roisin	86220
MURNANE, Graham	87840
EARNSHAW, Mark	75270
ALLAN, Jamie	90950
GORDON, Naoise	74290
McKAY, Jacqueline	95990
JONES, Keith	72980
BROWN, James	126270
MAIN, Malcolm	122920
DeZEEUW, Rick	125950
TEN BRINK, Carsten	95280
WALLER, Stephen	0136010
WATSON, Craig	143030
DRYSDALE, Douglas	143820
CORET, Sophie	144560
D'ARCY, Julia	143810
ANDERSON, Angela	078500

File Ref: P40798-/GUYF/MCM

11 May 2005

MURGITROYD
& COMPANY
European Patent and Trade Mark Attorneys
ABERDEEN · BELFAST · DUBLIN
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MUNICH · MÜNSTER · NICE

CONFIRMATION COPY

Your Ref: - - -
Our Ref: P40798-/GUYF/MCM

EPO - Munich
29
12. Mai 2005

European Patent Office
D-80298 Munich
Germany

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CONFIRMATION COPY VIA COURIER

Dear Sirs

European Patent Application No 03800355.4
EPO Phase of International Application PCT/US2003/041619
Exelixis, Inc.
IP Title: FLJ10607 as Modifier of the Axin Pathway and Methods of Use

We enclose the following documents:-

1. EPO Form 1200 (5 pages + supplementary sheet).

2. Sequence listing in computer readable form (via courier only). The information recorded on the data carrier is identical to the written sequence.

3. Fee Sheet instructing debit of EUR 3695 from deposit account number 2805 0177 in respect of National Basic fee, Search Fee (Less 20%), seven Designation Fees in respect of all Contracting States, and Extension Fees in respect of Lithuania, Albania, Latvia and The former Yugoslav Republic of Macedonia, Claims Fees for claims 11 to 25, Examination fee and Additional Copies Fee.

4. EPO Form 1037 for receipt and return.

The originally filed Request Form, Description, Claims, Abstract, Drawings and Priority Document will already be in your possession via the International Bureau.

Yours faithfully
for Murgitroyd & Company

MALCOLM MAIN
malcolm.main@murgitroyd.com

Enc: As Above

INTERNATIONAL PATENTS, TRADE MARKS, REGISTERED DESIGNS AND COPYRIGHT
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Filing Office Munich

File Number : 03800355.4

EPO - Munich
29
12. Mai 2005

Date of Receipt : .....

SEQL disc (EP)

EPO - Munich
29
12. Mai 2005

Sent on : .....

To : Peter Keasberry
(Den Haag - Room S13C20)

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GENEVA

With the compliments of the PCT Document Communication Section

*****

Avec les compliments de la Section de la communication des documents du PCT

☐..... ISR Published on .....

☐..... ISR waiting for publication

☐..... ISR will be published on .....

☒..... ISR not received, ISA US

☐..... P. Doc. not received

☐ Has been requested pursuant to Rule 17.1(b) PCT

☐ Has not been requested pursuant to Rule 17.1(b) PCT

☐..... IPER not available, IPEA .....

☐..... IPER translation not yet available

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34, Chemin des Colombettes
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SWITZERLAND

Datum/Date
07-06-2005

Zeichen/Ref./Réf.	Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°.
P40798-GUYF/MCM	PCT/US0341619 - EP/03800355.4-1212 / ISA US
Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire: Exelixis, Inc.	

For the aforementioned international application, you are hereby kindly requested to forward to the EPO in its capacity as designated/elected Office:

(X) a) the publication of the international search report (Art. 20 PCT)

( ) b) the copy of the international preliminary examination report (Art. 36(3)(a) PCT)

( ) c) the copy (copies) of the priority document(s). If any document is not available and ISA is not EP, please indicate below whether the receiving Office has been requested to transmit the document to the International Bureau (Form PCT/RO/101, Box VI; Rule 17.1(b) PCT).

( ) ..... .

RECEIVING SECTION

Answer of the International Bureau [IB]:

( ) The requested item [a), b) or c)] is not available with the IB.

Reason: ..... .

For priority documents [c)] with ISA not EP:
( ) The applicant has requested the receiving Office to issue a priority document (Rule 17.1(b) PCT) but the IB has not received it.

The International Bureau

EPO-DG 1

21. 06. 2005

TEAM 14